EXHIBIT AB

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/661,165	09/11/2003	Ravinder S. Dhallan	543312000420	7501

25226 7590 01/30/2007

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EXAMINER	
WHISENANT, ETHAN C	
ART UNIT	PAPER NUMBER
1634	

SHORTENED STATUTORY PERIOD OF RESPONSE	MAIL DATE	DELIVERY MODE
3 MONTHS	01/30/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Office Action Summary	Application No.	Applicant(s)	
	10/661,165	DHALLAN, RAVINDER S.	
	Examiner	Art Unit	
	Ethan Whisenant, Ph.D.	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE ___ MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12 December 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-6,8-12,14-16,18-83,87-102,132-146,148-152,181-196 and 201-208* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-6,8-12,14-16,18-24. 26-30, 32-34, 36-39, 41, 43-52, 56-83, 87-102,132-146,148-152, 181-196 and 201-208* is/are rejected.

7)☒ Claim(s) *25,31,35,40,42 and 53-55* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *11 September 2003* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

NON-FINAL ACTION

1. The applicant's response (filed 14 APR 03) has been entered. Following the entry of the claim amendment(s), **Claim(s) 1-6, 8-12, 14-16, 18-83, 87-102, 132-146, 148-152, 181-196, and 201-208** is/are pending. The **Finality** of the prior Office action has been withdrawn in order in impose a new grounds of rejection over newly discovered prior art i.e. Umansky et al. [US 2002/0119478(2002)] .

CLAIM OBJECTIONS

2. **Claim(s) 18-19, 58,64, 67-68, 83, 87 and 148** is /are objected to for the following minor informalities.

Applicant is advised that should **Claim 18** be found allowable, **Claim 19** will be objected to under 37 CFR 1.75 as being a substantial duplicate thereof. When two claims in an application are duplicates or else are so close in content that they both coverthe same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 706.03(k).

Claims 58 is objected to because of the phrase "has been added" Should not this phrase read "is added".

Claims 64 is objected to because of the phrase "was isolated" Should not this phrase read "is isolated".

Claims 67-68 are objected to because of the phrase "was determined" Should not this phrase read "is determined".

Claims 83 is objected to because of the phrase "was determined" Should not this phrase read "is determined".

Claims 87 is objected to because of the phrase "has been added" Should not this phrase read "is added".

Claims 148 is objected to because of the phrase "has been added" Should not this phrase read "is added".

35 USC § 112- 2nd Paragraph

3. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

CLAIM REJECTIONS under 35 USC § 112- 2ND PARAGRAPH

4. **Claim(s) 26, 80-82** is/are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 26 is indefinite because the phrase "the incorporation of a nucleotide in (c)" lacks proper antecedent basis in Claim 1.

Claim 80 is indefinite because the phrase "the portion of the 3' region" lacks proper antecedent basis in Claims 73, 74, 78 and/or 79.

Claim 81 is indefinite because the phrase "the portion of the 3' region" lacks proper antecedent basis in Claims 73, 74, 78, 79 and/or 80.

35 USC § 103

5. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> **(a)** A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

CLAIM REJECTIONS UNDER 35 USC § 103

6. Claim(s) 1-4, 8, 52, 56-57, 152, 181-188, 202-205 and 207-208 is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Umansky et al. [US 2002/0119478(2002)] in view of Saiki et al. [NEJM 46(2) : 301-302 (2000)].

Claim 1 is drawn to a method for detecting the presence or absence of a fetal chromosomal abnormality which method comprises quantitating the relative amount of the alleles at a heterozygous locus of interest wherein said heterozygous locus of interest has been determined by determining the sequence of the alleles at a locus of interest from template DNA obtained from a pregnant female, wherein said relative amount is expressed as a ratio, wherein said ratio indicates the presence or absence of a fetal chromosomal abnormality, and wherein said template DNA comprises a mixture of maternal DNA and fetal DNA.

Umansky et al. teach a method of detecting the presence or absence of a fetal chromosomal abnormality which method comprises all of the limitations recited in Claim 1 except these authors do not teach expressing the relative amounts of the alleles as a ratio. In fact, Umansky is silent as regards how to express the relative amount of the alleles at the heterogeneous locus of interest. Umansky does teach beginning on p. 10 :

> "Many diseases inherited by the fetus will be easily detectable by analysis of the mother's urine DNA. These include Marfan Syndrome, Sickle Cell Anemia, Tay Sachs Disease, and a group of neurodegenerative disorders, including Huntington's Disease, Spinocerebellar Ataxia 1, Machado-Joseph Disease, Dentatorubraopallidoluysian Atrophy, and others that affect the fetus and newborn. Urine DNA analysis can detect the presence of the mutant gene inherited from the father. Also, if the mother's genome bears a mutation, the test can help determine whether a normal version of the gene has been inherited from the father. "

However, Saiki et al. do teach a method diagnosing sickle cell anemia wherein the relative amount of the alleles at a heterozygous locus of interest are determined and results of the assay are expressed as a ratio (i.e. β^a β^a or β^a β^s or β^s β^s). In light of these teachings and absent an unexpected result it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method of Umansky et al. wherein the results of the assay are expressed as a ratio as taught by Saiki et al. The ordinary artisan would have been motivated to make the modification of Umansky in order to quickly and succinctly relay the results of their prenatal tests to individual reading the results of said test(s). As regards the limitation which reads wherein said template DNA comprises a mixture of maternal DNA and fetal DNA. This limitation is inherent to Umansky in that both maternal and fetal free DNA present in the maternal blood stream will pass over into the maternal urine (i.e. maternal urine comprises both free fetal DNA as well as free maternal DNA)

Umansky et al. or Saiki et al. teach the limitations recited in **Claims 2-4, and 8, 52 and 56-57**.

Claim 152 is drawn to a method for detecting the presence or absence of a fetal chromosomal abnormality which method comprises two steps, To begin, the sequence of alleles of a locus of interest from template DNA is determined wherein the template DNA comprises a mixture of maternal DNA and fetal DNA and wherein said mixture is obtained from a pregnant female. Finally, the relative amount of the alleles at a heterozygous locus of interest that was identified from the locus of interest of step (a) is quantitated wherein the relative amount is expressed as a ratio and wherein the said ratio indicates the presence or absence of a fetal chromosomal abnormality.

Umansky et al. teach a method of detecting the presence or absence of a fetal chromosomal abnormality which method comprises all of the limitations recited in Claim 152 except these authors do not teach expressing the relative amounts of the alleles as a ratio. In fact, Umansky is silent as regards how to express the relative amount of the alleles at the heterogeneous locus of interest. Umansky does teach beginning on p. 10 :

> "Many diseases inherited by the fetus will be easily detectable by analysis of the mother's urine DNA. These include Marfan Syndrome, Sickle Cell Anemia, Tay Sachs Disease, and a group of neurodegenerative disorders, including Huntington's Disease, Spinocerebellar Ataxia 1, Machado-Joseph Disease, Dentatorubraopallidoluysian Atrophy, and others that affect the fetus and newborn. Urine DNA analysis can detect the presence of the mutant gene inherited from the father. Also, if the mother's genome bears a mutation, the test can help determine whether a normal version of the gene has been inherited from the father. "

However, Saiki et al. do teach a method diagnosing sickle cell anemia wherein the relative amount of the alleles at a heterozygous locus of interest are determined and results of the assay are expressed as a ratio (i.e. β^a β^a or β^a β^s or β^s β^s). In light of these teachings and absent an unexpected result it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method of Umansky et al. wherein the results of the assay are expressed as a ratio as taught by Saiki et al. The ordinary artisan would have been motivated to make the modification of Umansky in order to quickly and succinctly relay the results of their prenatal tests to individual reading the results of said test(s). As regards the limitation which reads wherein said template DNA comprises a mixture of maternal DNA and fetal DNA. This limitation is inherent to Umansky in that both maternal and fetal free DNA present in the maternal blood stream will pass over into the maternal urine (i.e. maternal urine comprises both free fetal DNA as well as free maternal DNA).

Claim 181 is drawn to an embodiment of the method of Claim 1 wherein the sample is selected from a defined group which includes urine.

Umansky et al. teach this limitation.

Claim 182 is drawn to an embodiment of the method of Claim 181

wherein the sample is blood. **Claim 183** is drawn to an embodiment of the method of Claim 182 wherein the sample is blood plasma. **Claim 184** is drawn to an embodiment of the method of Claim 182 wherein the sample is blood serum.

Admittedly, Umansky et al. do not explicitly teach these limitations. However, these limitations would have been *prima facie* obvious to the ordinary artisan at the time of the invention, in the absence of an unexpected result in light of the teachings in Umansky et al. Umansky et al. teach that both maternal and fetal free DNA are present in the blood of a pregnant female before some portion thereof passes over the kidney barrier into the pregnant female's urine. In light of this teaching it would have been, absent an unexpected result, *prima facie* obvious to the ordinary artisan at the time of the invention to analyze the cell free portion of the blood (i.e. plasma or serum) from the pregnant female rather than or, in addition to, the urine sample using the method of Saiki et al. Also please note that it was well known prior to the instant invention to analyze free fetal DNA present in a pregnant female's plasma or serum, see Lo et al. [The Lancet 350 :485-487(1997)]. Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

Claim 185 is drawn to an embodiment of the method of Claim 8 wherein the template DNA is obtained from a source selected from a defined group which includes urine.

Umansky et al. teach this limitation.

Claim 186 is drawn to an embodiment of the method of Claim 185 wherein the sample is blood. **Claim 187** is drawn to an embodiment of the method of Claim 186 wherein the sample is blood plasma. **Claim 188** is drawn to an embodiment of the method of Claim 186 wherein the sample is blood serum.

Admittedly, Umansky et al. do not explicitly teach these limitations. However, these limitations would have been *prima facie* obvious to the ordinary artisan at the time of the invention, in the absence of an unexpected result in light of the teachings in Umansky et al. Umansky et al. teach that both maternal and fetal free DNA are present in the blood of a pregnant female before some portion thereof passes over the kidney barrier into the pregnant female's urine. In light of this teaching it would have been, absent an unexpected result, *prima facie* obvious to the ordinary artisan at the time of the invention to analyze the cell free portion of the blood (i.e. plasma or serum) from the pregnant female rather than or, in addition to, the urine sample using the method of Saiki et al. Also please note that it was well known prior to the instant invention to analyze free fetal DNA present in a pregnant female's plasma or serum, see Lo et al. [The Lancet 350 :485-487(1997)]. Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

Claim 202 is drawn to an embodiment of the method of Claim 152 wherein the template DNA is obtained from a source selected from a defined group which includes urine.

Umansky et al. teach this limitation.

Claim 203 is drawn to an embodiment of the method of Claim 202 wherein the sample is blood. **Claim 204** is drawn to an embodiment of the method of Claim 203 wherein the sample is blood plasma. **Claim 205** is drawn to an embodiment of the method of Claim 203 wherein the sample is blood serum.

Admittedly, Umansky et al. do not explicitly teach these limitations. However, these limitations would have been *prima facie* obvious to the ordinary artisan at the time of the invention, in the absence of an unexpected result in light of the teachings in Umansky et al. Umansky et al. teach that both maternal and fetal free DNA are present in the blood of a pregnant female before some portion thereof passes over the kidney barrier into the pregnant female's urine. In light of this teaching it would have been, absent an unexpected result, *prima facie* obvious to the ordinary artisan at the time of the invention to analyze the cell free portion of the blood (i.e. plasma or serum) from the pregnant female rather than or, in addition to, the urine sample using the method of Saiki et al. Also please note that it was well known prior to the instant invention to analyze free fetal DNA present in a pregnant female's plasma or serum, see Lo et al. [The Lancet 350 :485-487(1997)]. Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

Claim 207 is drawn to an embodiment of the method of Claim 1 or 152 wherein said mixture comprises at least about 15% fetal DNA. **Claim 208** is drawn to an embodiment of the method of Claim 1 or 152 wherein said mixture comprises a maximum of about 98-99%.

Umansky et al. teaches that at least some portion of the DNA in a pregnant female's urine is fetal DNA. Claims 207-208 are very broad and encompass any percentage of fetal DNA present in said mixture.

7. Claim(s) 5-6, 20-24, 26-30, 32-34, 36-39, 41 and 44-51 is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Umansky et al. [US 2002/0119478(2002)] in view of Saiki et al. [NEJM 319 : 537-541 (1988)] as applied against Claim 1 and further in view of Jones et al. [US 2003/0082576 (2003)].

Claim 5 is drawn to an embodiment of the method of Claim 1 wherein alleles of multiple loci of interest are sequenced and their relative amounts are expressed as a ratio.

Umansky et al. in view of Saiki et al. reasonably suggest a method of detecting the presence or absence of a fetal chromosomal abnormality comprising all of the limitations recited in Claim 5 except these authors do not teach sequencing multiple loci of interest. However, as evidenced by at least Jones et al. such multiplexing was routine in the art at the time of the invention. In light of these findings, and absent an unexpected, it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to modify the method reasonably suggested by the combination of Umansky et al. in view of Saiki et al. wherein multiple loci on multiple chromosomes are analyzed. The ordinary artisan would have been motivated to make the modification recited above in order to analyze multiple different disease/disorder associated genes taught by Umansky et al. - see paragraph [0121] - for mutations simultaneously (i.e. in a single assay).

Claim 6 is drawn to an embodiment of the method of Claim 6 wherein alleles of multiple loci of interest are on multiple chromosomes.

Jones et al. teach this limitation. See, for example, the second column on p. 1926.

Claim 20 is drawn to an embodiment of the method of Claim 1 wherein the DNA sample is analyzed using a particular assay. **Claim 21** is drawn to an embodiment of the method of Claim 1 wherein the DNA sample is analyzed using a multiplexed version of said particular assay.

Admittedly, none of Umansky et al or Saiki et al. teach the assay recited in Claims 20 and 21. However, Jones et al. do teach the exact assay recited in Claims 20-21. Therefore, absent an unexpected result, it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method reasonably suggested by Umansky et al or Saiki et al. wherein the assay of Jones et al. is used in place of the assay taught by the combination of Umansky et al in view of Saiki et al. Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

Claim 22 is drawn to an embodiment of the method of Claim 20 or 21 wherein the incorporation of a nucleotide in step (c) is by a polymerase selected from a defined group which includes Taq polymerase.

Jones et al. teach this limitation wherein these authors teach utilizing AmpliTaq®. Please note that AmpliTaq® is a type of Taq polymerase. In support of

this position, see the Applied Biosystems description of AmpliTaq® and the paper by Bradley, A.F. [Pure & Applied Chemistry 68 (10) : 1907-1912 (1996)].

Umansky et al., Saiki et al. or Jones et al. teach the limitations recited in **Claims 23-24, 26-30, 32-34, 36-39, 41 and 44-51.**

8. **Claim(s) 9-12, 14-16, 18-19 and 189** is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Umansky et al. [US 2002/0119478(2002)] in view of Saiki et al. [NEJM 319 : 537-541 (1988)] as applied against Claims 4 above and further in view of Kiessling [US 5,618,664(1998)].

Claim 9 is drawn to an embodiment of the method of Claim 4 wherein the sample is mixed with an agent that inhibits cell lysis wherein the agent is selected from a defined group which includes a cell lysis inhibitor.

Umansky et al. in view of Saiki et al. reasonably suggest a method of detecting the presence or absence of a fetal chromosomal abnormality comprising all of the limitations recited in Claim 9 except these authors do not teach mixing their sample with an agent that inhibits cell lysis wherein the agent is selected from a defined group which includes a cell lysis inhibitor. However as evidenced by Kiessling the addition of an agent to a biological sample which simultaneously inhibits cell lysis and disinfects said sample was well known prior to the instant invention. Therefore, absent an unexpected, it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to modify the method reasonably suggested by the combination of Umansky et al. in view of Saiki et al. wherein an agent which inhibits cell lysis is mixed with the urine sample prior to its analysis. The ordinary artisan would have been motivated to make the modification recited above in order to disinfect the urine sample for the protection of the laboratory personnel handling and processing the sample during its analysis.

Claim 10 is drawn to an embodiment of the method of Claim 9 wherein said agent is a cell lysis inhibitor.

Kiessling teach this limitation wherein this authors teach that their fixative lyses red blood cells (i.e. RBCs) while fixing (i.e. inhibiting the lysis of) white blood cells (i.e. WBCs).

Claim 11 is drawn to an embodiment of the method of Claim 10 wherein said cell lysis inhibitor is selected from a defined group which includes derivatives of formaldehyde.

Kiessling teach this limitation wherein these author teach the use of paraformaldehyde.

Claim 12 is drawn to an embodiment of the method of Claim 9 wherein the template DNA is obtained from blood.

While Umansky et al. do not explicitly teach this limitation, this limitation would have been *prima facie* obvious to the ordinary artisan at the time of the invention, in the absence of an unexpected result in light of the teachings in Umansky et al. Umansky et al teach that both maternal and fetal free DNA are present in the blood of a pregnant female before some portion thereof passes over the kidney barrier into the pregnant female's urine. In light of this teaching it would have been, absent an unexpected result , *prima facie* obvious to the ordinary artisan at the time of the invention to analyze the cell free portion of a maternal blood sample (i.e. plasma or serum) from the pregnant female treated according to the method of Kiessling rather than or, in addition to, the urine sample treated according to the method of Kiessling for a chromosomal abnormality using the method of Saiki et al. The analysis of blood plasma and/or serum from pregnant females was well known . See for example Lo et al. [Lancet 350: 485-487 (1997).

Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the

absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

Claim 14 is drawn to an embodiment of the method of Claim 12 wherein the blood sample is taken from a human pregnant female sometime during her pregnancy. **Claim 15** is drawn to an embodiment of the method of Claim 12 wherein said template DNA is obtained from the plasma of said blood. **Claim 16** is drawn to an embodiment of the method of Claim 12 wherein said template DNA is obtained from the serum of said blood.

Admittedly, Umansky et al. do not explicitly teach these limitations. However, these limitations would have been *prima facie* obvious to the ordinary artisan at the time of the invention, in the absence of an unexpected result in light of the teachings in Umansky et al. Umansky et al. teach that both maternal and fetal free DNA are present in the blood of a pregnant female before some portion thereof passes over the kidney barrier into the pregnant female's urine. In light of this teaching it would have been, absent an unexpected result, *prima facie* obvious to the ordinary artisan at the time of the invention to analyze the cell free portion of the blood (i.e. plasma or serum) from the pregnant female rather than or, in addition to, the urine sample using the method of Saiki et al. Also please note that it was well known prior to the instant invention to analyze free fetal DNA present in a pregnant female's plasma or serum, see Lo et al. [The Lancet 350 :485-487(1997)]. Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

Claim 18 is drawn to an embodiment of the method of Claim 15 or 16 wherein prior to determining the sequence of the locus of interest on fetal DNA the sequence of the locus of interest on maternal template DNA is determined. **Claim 19** is drawn to an embodiment of the method of Claim 15 or 16 wherein prior to determining the sequence of the locus of interest on fetal DNA the sequence of the locus of interest on maternal (? paternal) template DNA is determined.

As regards the limitations present in Claims 18-19, it must be noted that none of Umansky et al. or Saiki et al. teach testing the maternal and/or paternal DNA prior to testing the fetal DNA. Saiki et al. do teach testing both maternal and paternal DNA just

not prior to testing the fetal DNA. However, absent an unexpected result it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to modify the method reasonably suggested by the combination of Umansky et al. in view of Saiki et al. wherein maternal and/or paternal DNA is tested prior to or simultaneously with the fetal DNA. It would have been *prima facie* obvious to an ordinary practitioner to switch the order of ingredient addition see MPEP 2144.04 which refers to *In re Gibson*, 39 F.2d 975,5 USPQ 230 (CCPA 1930). Selection of any order of mixing ingredients is *prima facie* obvious".

Claim 189 is drawn to an embodiment of the method of Claim 11 wherein said cell lysis inhibitor is selected from a defined group which includes gluteraldehyde, formaldehyde and formalin.

Kiessling teach these limitations, See Column 5, lines 36-56 of Kiessling wherein Kiessling teaches :

> "As used herein, the term "fixative" refers to an agent that is capable of preserving the structure of a biological molecule. Fixatives that are useful for the purposes of the instant invention include the well-known fixatives that are commonly used for flow cytometry and tissue fixation applications. (See, e.g., Lifson, J., et al., J. Immunol., Methods 86:143-149 (1986), the entire contents of which are incorporated by reference). These include, for example, paraformaldehyde, **formaldehyde and glutaraldehyde**. It is believed that fixatives, such as paraformaldehyde, act by crosslinking proteins, with the resulting crosslinked products stabilizing the cellular ultrastructure. (see, e.g., Aloisio, C., and Nicholson, J., J. Immunol. Methods. 128:281-285 (1990), the entire contents of which are incorporated by reference, and references cited therein). Also provided herein is a method for evaluating (screening) aldehyde and non-aldehyde (e.g., Streck's Tissue Fixative ("STF")) fixatives for use in accordance with the methods of the invention. (See e.g., Science 260:976-979 (1993), the entire contents of which are incorporated herein by reference)."

Also note in Kiessling , the paragraph bridging Columns 5-6 where Kiessling teaches

> "In the preferred embodiments, the fixative solution contains between about 2% and about 10% paraformaldehyde. However, the preferred "disinfecting concentration" for a particular fixative is prescribed, at least in part, by the nature of the biological fluid. Thus, for blood samples in which it is desirable to simultaneously disinfect the sample, lyse the red blood cells and preserve the leukocytes for analysis, the preferred fixative is paraformaldehyde having a disinfecting concentration of between about 2% and about 4%. This is because it is generally believed that paraformaldehyde concentrations less

> than 2% are not immediately (i.e., within 5-10 minutes) disinfecting, while concentrations greater than 4% fix (not lyse) the red blood cells."

It should be noted that formalin is simply a dilute aqueous solution of formaldehyde.

9. **Claim(s) 43** is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Umansky et al. [US 2002/0119478(2002)] in view of Saiki et al. [NEJM 319 : 537-541 (1988)] and Jones et al. [US2003/0082576 (2003)] as applied against Claim 20 and 21 above and further in view of MacLeod et al. [US 6,221,600 (2001)] and Polisson [US 5,098,839 (1992)].

Claim 43 is drawn to an embodiment of the method of Claim 1 wherein the restriction enzyme recognition site is for a restriction enzyme selected from a defined group which includes : BsaJ I and Bssk I and Dde I and EcoNI and Fnu4H I and Hinf I and ScrF I.

Umansky et al. in view of Saiki et al. and Jones et al. reasonably suggest a method comprising all of the limitations of Claim 43 except these authors do not teach restriction enzymes recited. However, as evidenced by MacLeod et al. and Polisson the restriction enzymes recited well known prior to the instant invention. Therefore, absent an unexpected result, it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method reasonably suggested by the combination of Umansky et al. in view of Saiki et al. and Jones et al. wherein the Type IIS restriction enzymes taught by MacLeod et al. and Polisson are used rather the Type IIS restriction enzymes taught in Jones et al. Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

10. Claim(s) 58-68, 87-102, 190-196, 201 is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Umansky et al. [US 2002/0119478(2002)] in view of Kiessling [US 5,618,664(1998)].

Claim 58 is drawn to a method which comprises determining the sequence of a locus of interest on free fetal DNA from a sample comprising free fetal DNA wherein an agent that inhibits cell lysis has been added to said sample to inhibit the lysis of cells present therein, wherein said agent is selected from a defined group which includes membrane stabilizer, cross-linker, and cell lysis inhibitor.

Umansky et al. teach a method of determining the sequence of a fetal locus of interest (i.e. diagnosing certain genetic diseases – see paragraph [0121] - from a maternal urine sample which utilizes free fetal DNA in sample comprising both maternal DNA and fetal DNA. As regards the limitation which reads "determining the sequence of a fetal locus of interest", this limitation is present in Umansky et al. in that when Umansky et al. teach diagnosing certain genetic diseases – see paragraph [0121] - the sequence at the locus of interest of the fetal DNA sequence is determined. In summary, Umansky et al. teach a method comprising all of the limitations of Claim 58 except these authors do not teach adding an agent that inhibits cell lysis to their maternal urine samples. However, Kiessling does teach adding an agent to a biological sample which simultaneously disinfects and inhibits the lysis of cells therein. Therefore, absent an unexpected result, it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method of Umansky et al. wherein the maternal urine sample is treated according to the method of Kiessling. The ordinary artisan would have been motivated to make the modification recited above in order to reduce the risk of viral infection for those laboratory personnel performing the assay(s) of Umansky et al. It is noted that the fixative(s) taught by Kiessling lyses RBCs while fixing (i.e. inhibiting the lysis of) WBCs.

Claim 59 is drawn to an embodiment of the method of Claim 58 wherein the sample is selected from a defined group which includes urine.

Both of Umansky et al. and Kiessling teach this limitation. See for example the first paragraph in Column 5 of Kiessling.

Claim 60 is drawn to an embodiment of the method of Claim 59 wherein the template DNA is obtained from blood.

While Umansky et al. do not explicitly teach this limitation, this limitation would have been *prima facie* obvious to the ordinary artisan at the time of the invention, in the absence of an unexpected result. Umansky et al teach that both maternal and fetal free DNA are present in the blood of a pregnant female before some portion thereof

passes over the kidney barrier into the pregnant female's urine. In light of this teaching it would have been, absent an unexpected result , *prima facie* obvious to the ordinary artisan at the time of the invention to analyze the cell free portion of a maternal blood sample (i.e. plasma or serum) from the pregnant female treated according to the method of Kiessling rather than or, in addition to, the urine sample treated according to the method of Kiessling for a chromosomal abnormality using the method of Saiki et al. The analysis of blood plasma and/or serum from pregnant females was well known, see for example Lo et al. [Lancet 350: 485-487 (1997).

Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

Claim 61 is drawn to an embodiment of the method of Claim 58 wherein the sample comprises free maternal template DNA and free fetal template DNA.

Admittedly, none of Umansky et al. or Kiessling explicitly teach this limitation. However, this limitation is considered inherent to Umansky et al. in that the blood of a pregnant female will normally comprises both maternal free DNA and fetal free DNA which results from the leakage of cellular DNA into the maternal blood following cell death. Some portion of this free DNA then passes over the kidney barrier into the urine.

Umansky et al. or Kiessling teach the limitations recited in **Claim 62-64**

Claim 65 is drawn to an embodiment of the method of Claim 60 wherein

said template DNA is obtained from the plasma of said blood. **Claim 66** is drawn to an embodiment of the method of Claim 60 wherein said template DNA is obtained from the serum of said blood.

Admittedly, Umansky et al. do not explicitly teach these limitations. However, these limitations would have been *prima facie* obvious to the ordinary artisan at the time of the invention, in the absence of an unexpected result in light of the teachings in Umansky et al. Umansky et al. teach that both maternal and fetal free DNA are present in the blood of a pregnant female before some portion thereof passes over the kidney barrier into the pregnant female's urine. In light of this teaching it would have been, absent an unexpected result, *prima facie* obvious to the ordinary artisan at the time of the invention to analyze the cell free portion of the blood (i.e. plasma or serum) from the pregnant female rather than or, in addition to, the urine sample using the method of Saiki et al. Also please note that it was well known prior to the instant invention to analyze free fetal DNA present in a pregnant female's plasma or serum, see Lo et al. [The Lancet 350 :485-487(1997)]. Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

Claim 67 is drawn to an embodiment of the method of Claim 58 wherein prior to determining the sequence of the locus of interest on fetal DNA the sequence of the locus of interest on maternal template DNA is determined. **Claim 68** is drawn to an embodiment of the method of Claim 58 wherein prior to determining the sequence of the locus of interest on fetal DNA the sequence of the locus of interest on paternal template DNA is determined.

As regards the limitations present in Claims 67-68, it must be noted (Official Notice) that while none of Umansky et al. or Kiessling teach testing the maternal and/or paternal DNA prior to testing the fetal DNA, it would have been, absent an unexpected result *prima facie* obvious to one of ordinary skill in the art at the time of the invention to modify the method reasonably suggested by the combination of Umansky et al. in view of Kiessling wherein maternal and/or paternal DNA is tested prior to, or simultaneously with the fetal DNA.

Claim 87 is drawn to a method for preparing a sample for analysis comprising isolating free nucleic acid from a sample that contains nucleic acid wherein an agent that inhibits cell lysis is added to the sample to inhibit the lysis of cells, if cells are present wherein the agent is selected from a defined group which includes a cell lysis inhibitor.

Umansky et al. teach a method of preparing a sample for analysis which comprises all of the limitations recited in Claim 87 except these authors do not teach adding an agent that inhibits cell lysis is added to the sample to inhibit the lysis of cells, if cells are present wherein the agent is selected from a defined group which includes a cell lysis inhibitor. However as evidenced by Kiessling the addition of an agent to a biological sample which simultaneously inhibits cell lysis and disinfects said sample was well known prior to the instant invention. Therefore, absent an unexpected result, it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to modify the method taught by Umansky et al. wherein an agent which inhibits cell lysis is mixed with the urine sample prior to its analysis. The ordinary artisan would have been motivated to make the modification recited above in order to disinfect the urine sample for the protection of the laboratory personnel performing the assay(s) disclosed by Umansky et al.

Umansky et al. or Kiessling teach the limitations recited in **Claims 88-89.**

Claim 90 is drawn to an embodiment of the method of Claim 87 wherein

the sample is obtained from a source selected from a defined group which includes urine.

Umansky et al. teach this limitation. See at least the abstract.

Claim 91 is drawn to an embodiment of the method of Claim 90 wherein said sample is blood. **Claim 92** is drawn to an embodiment of the method of Claim 91 wherein said blood is from a pregnant female. **Claim 93** is drawn to an embodiment of the method of Claim 92 wherein said blood is from a pregnant female at some point during her pregnancy. **Claim 94** is drawn to an embodiment of the method of Claim 93 wherein said template DNA is obtained from the plasma of said blood.

While Umansky et al. do not explicitly teach these limitations, these limitations would have been *prima facie* obvious to the ordinary artisan at the time of the invention, in the absence of an unexpected result in light of the teachings in Umansky et al. Umansky et al teach that both maternal and fetal free DNA are present in the blood of a pregnant female before some portion thereof passes over the kidney barrier into the pregnant female's urine. In light of this teaching it would have been, absent an unexpected result , *prima facie* obvious to the ordinary artisan at the time of the invention to analyze the cell free portion of a maternal blood sample (i.e. plasma or serum) from the pregnant female treated according to the method of Kiessling rather than or, in addition to, the urine sample treated according to the method of Kiessling for a chromosomal abnormality using the method(s) disclosed by Umansky et al. The analysis of blood plasma and/or serum from pregnant females was well known . See for example Lo et al. [Lancet 350: 485-487 (1997).

Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when

combined for their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

Claim 95 is drawn to an embodiment of the method of Claim 87 wherein the agent is a cell lysis inhibitor.

Kiessling teach this limitation. See for example, the first paragraph in Column 5 of Kiessling

Claim 96 is drawn to an embodiment of the method of Claim 87 wherein the cell lysis inhibitor is selected from a defined group which includes derivatives of formaldehyde.

Kiessling teaches this limitation. See for example Column 6, line 3-13.

Claim 97 is drawn to an embodiment of the method of Claim 87 wherein said cell lysis inhibitor is formalin.

Kiessling teach this limitations, See Column 5, lines 36-56 of Kiessling wherein Kiessling teaches :

> "As used herein, the term "fixative" refers to an agent that is capable of preserving the structure of a biological molecule. Fixatives that are useful for the purposes of the instant invention include the well-known fixatives that are commonly used for flow cytometry and tissue fixation applications. (See, e.g., Lifson, J., et al., J. Immunol., Methods 86:143-149 (1986), the entire contents of which are incorporated by reference). These include, for example, paraformaldehyde, **formaldehyde and glutaraldehyde**. It is believed that fixatives, such as paraformaldehyde, act by crosslinking proteins, with the resulting crosslinked products stabilizing the cellular ultrastructure. (see, e.g., Aloisio, C., and Nicholson, J., J. Immunol. Methods. 128:281-285 (1990), the entire contents of which are incorporated by reference, and references cited therein). Also provided herein is a method for evaluating (screening) aldehyde and non-aldehyde (e.g., Streck's Tissue Fixative ("STF")) fixatives for use in accordance with the methods of the invention. (See e.g., Science 260:976-979 (1993), the entire contents of which are incorporated herein by reference)."

Also note in Kiessling , the paragraph bridging Columns 5-6 where Kiessling teaches

> "In the preferred embodiments, the fixative solution contains between about

> 2% and about 10% paraformaldehyde. However, the preferred "disinfecting concentration" for a particular fixative is prescribed, at least in part, by the nature of the biological fluid. Thus, for blood samples in which it is desirable to simultaneously disinfect the sample, lyse the red blood cells and preserve the leukocytes for analysis, the preferred fixative is paraformaldehyde having a disinfecting concentration of between about 2% and about 4%. This is because it is generally believed that paraformaldehyde concentrations less than 2% are not immediately (i.e., within 5-10 minutes) disinfecting, while concentrations greater than 4% fix (not lyse) the red blood cells."

It should be noted that formalin is simply a dilute aqueous solution of formaldehyde.

As regards the particular formalin concentrations recited in **Claims 98-99** The applicant should note that where the general conditions of a claim are disclosed in the prior art, it is not inventive, in the absence of an unexpected result, to discover the optimum or workable ranges by routine experimentation. *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955).

Umansky et al. and/or Kiessling teach the limitations recited in **Claims 100.**

Claim 101 is drawn to an embodiment of the method of Claim 100 wherein the centrifugation step is performed with the centrifuge braking power set to zero.

Admittedly , neither of Umansky et al. or Kiessling teach this limitation. However, it must be noted (Official Notice) that it was routine in the art to perform centrifugation step(s) wherein the centrifuge braking power set to zero. This is often done in steps involving DNA/RNA extraction with organic solvents in order to prevent disruption of the interface between the organic phase and the aqueous phase.

Claim 102 is drawn to an embodiment of the method of Claim 100 wherein the centrifugation step is performed at a particular speed which includes 0 to greater than 8000rpm.

Umansky et al. teach this limitation wherein these authors teach precipitating the

DNA and then collecting the precipitate via centrifugation at 10,000xg for 5 minutes. See paragraph [0187].

Claim 190 is drawn to an embodiment of the method of Claim 58 wherein the sample is obtained from a pregnant female. **Claim 191** is drawn to an embodiment of the method of Claim 190 wherein the pregnant female is a human. **Claim 192** is drawn to an embodiment of the method of Claim 191 wherein the sample is selected from a defined group which includes urine.

Umansky et al. teach this limitation. See at least the abstract.

Claim 193 is drawn to an embodiment of the method of Claim 192 wherein said sample is blood. **Claim 194** is drawn to an embodiment of the method of Claim 193 wherein the free fetal DNA is obtained from the plasma from said blood. **Claim 195** is drawn to an embodiment of the method of Claim 193 wherein the free fetal DNA is obtained from the serum from said blood.

Admittedly, Umansky et al. do not explicitly teach these limitations. However, these limitations would have been *prima facie* obvious to the ordinary artisan at the time of the invention, in the absence of an unexpected result in light of the teachings in Umansky et al. Umansky et al. teach that both maternal and fetal free DNA are present in the blood of a pregnant female before some portion thereof passes over the kidney barrier into the pregnant female's urine. In light of this teaching it would have been, absent an unexpected result, *prima facie* obvious to the ordinary artisan at the time of the invention to analyze the cell free portion of the blood (i.e. plasma or serum) from the pregnant female rather than or, in addition to, the urine sample using the method of Saiki et al. Also please note that it was well known prior to the instant invention to analyze free fetal DNA present in a pregnant female's plasma or serum, see Lo et al. [The Lancet 350 :485-487(1997)]. Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

Claim 196 is drawn to an embodiment of the method of Claim 63 wherein said cell lysis inhibitor is selected from a defined group which includes glutaraldehyde, formaldehyde and formalin. **Claim 201** is drawn to an embodiment of the method of Claim 96 wherein said cell lysis inhibitor is selected from a defined group which includes glutaraldehyde, formaldehyde and formalin.

Kiessling teach this limitations, See Column 5, lines 36-56 of Kiessling wherein Kiessling teaches :

"As used herein, the term "fixative" refers to an agent that is capable of

> preserving the structure of a biological molecule. Fixatives that are useful for the purposes of the instant invention include the well-known fixatives that are commonly used for flow cytometry and tissue fixation applications. (See, e.g., Lifson, J., et al., J. Immunol., Methods 86:143-149 (1986), the entire contents of which are incorporated by reference). These include, for example, paraformaldehyde, **formaldehyde and glutaraldehyde**. It is believed that fixatives, such as paraformaldehyde, act by crosslinking proteins, with the resulting crosslinked products stabilizing the cellular ultrastructure. (see, e.g., Aloisio, C., and Nicholson, J., J. Immunol. Methods. 128:281-285 (1990), the entire contents of which are incorporated by reference, and references cited therein). Also provided herein is a method for evaluating (screening) aldehyde and non-aldehyde (e.g., Streck's Tissue Fixative ("STF")) fixatives for use in accordance with the methods of the invention. (See e.g.,Science 260:976-979 (1993), the entire contents of which are incorporated herein by reference)."

Also note in Kiessling , the paragraph bridging Columns 5-6 where Kiessling teaches

> "In the preferred embodiments, the fixative solution contains between about 2% and about 10% paraformaldehyde. However, the preferred "disinfecting concentration" for a particular fixative is prescribed, at least in part, by the nature of the biological fluid. Thus, for blood samples in which it is desirable to simultaneously disinfect the sample, lyse the red blood cells and preserve the leukocytes for analysis, the preferred fixative is paraformaldehyde aving a disinfecting concentration of between about 2% and about 4%. This is because it is generally believed that paraformaldehyde concentrations less than 2% are not immediately (i.e., within 5-10 minutes) disinfecting, while concentrations greater than 4% fix (not lyse) the red blood cells."

It should be noted that formalin is simply a dilute aqueous solution of formaldehyde.

11. Claim(s) 69-70 and 83 is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Umansky et al. [US 2002/0119478(2002)] in view of Kiessling [US 5,618,664(1998)] as applied above against Claim 58 and further in view of Saiki et al. [NEJM 319 : 537-541 (1988)].

Claim 69 is drawn to an embodiment of the method of Claim 58 wherein said locus of interest is a SNP. **Claim 70** is drawn to an embodiment of the method of Claim 58 wherein said locus of interest is a mutation.

Umansky et al. in view of Kiessling reasonably suggest a method comprising all of the limitations of Claim 69-70 except these authors do not teach an embodiment wherein the locus of interest is a SNP or a mutation. However, as evidenced by at least Saiki et al. the analysis of a the locus of interest that is a SNP or a mutation was well known prior to the instant invention. See at least for example the abstract. Therefore, absent an unexpected result, it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method reasonably suggested by the combination of Umansky et al. in view of Kiessling wherein the locus analyzed is a SNP or a mutation. Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

Claim 83 is drawn to an embodiment of the method of Claim 58 the sequence of said locus of interest is determined using a method selected from a defined group which includes dot blots.

Saiki et al. teach this limitation.

12. Claim(s) 71-82 is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Umansky et al. [US 2002/0119478(2002)] in view of Kiessling [US 5,618,664(1998)] as applied against Claim 58 above and further in view of Jones et al. [US 2003/0082576 (2003)].

Claim 71 is drawn to an embodiment of the method of Claim 58 wherein the sequence of multiple loci of interest is determined.

Umansky et al. or Kiessling reasonably suggest a method as recited in Claim 71 except these authors do not teach determining the sequence of multiple loci of interest. However, as evidenced by at least Jones et al. such multiplexing was routine in the art at the time of the invention. In light of these findings, and absent an unexpected, it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to modify the method reasonably suggested by the combination of Umansky et al. in view of Kiessling. wherein multiple loci on multiple chromosomes are analyzed. The ordinary artisan would have been motivated to make the modification recited above in order to analyze multiple different disease/disorder associated genes for mutations simultaneously (i.e. in a single assay).

Umansky et al., Saiki et al. or Jones et al. teach the limitations recited in **Claims 72-82.**

13. Claim(s) 132, 134-142 is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Umansky et al. [US 2002/0119478(2002)] in view of Saiki et al. [NEJM 46(2) : 301-302 (2000)] as applied against Claim 1 above and further in view of Chen et al. [Genome Research 10: 549-557(2000)].

Claim 132 is drawn to an embodiment of the method of Claim 1 wherein the DNA sample is analyzed using a particular assay.

Umansky et al. in view of Saiki et al. reasonably suggest a method for detecting the presence or absence of a fetal chromosomal abnormality comprising all of the limitations recited in Claim 132 except these authors do not teach the particular assay recited in Claim 132. However, Chen et al. do teach the exact assay recited in Claim 132 Therefore, absent an unexpected result it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method reasonably suggested by the combination of Umansky et al. in view of Saiki et al. wherein the method of Chen et al. is used in place of the assay(s) reasonably suggested by the

combination of Umansky et al. in view of Saiki et al. Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

Umansky et al., Saiki et al. and/or Chen et al. teach the limitations recited in Claims 134-142.

14. Claim(s) 133-142 and 206 is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Umansky et al. [US 2002/0119478(2002)] in view of Kiessling [US 5,618,664(1998)] as applied against Claim 58 above and further in view of Chen et al. [Genome Research 10: 549-557(2000)].

Claim 133 is drawn to an embodiment of the method of Claim 58 wherein the DNA sample is analyzed using a particular assay.

Umansky et al. in view of Kiessling reasonably suggest a method for determining the sequence of a locus of interest on free fetal DNA comprising all of the limitations recited in Claim 133 except these authors do not teach the particular assay recited in Claim 133. However, Chen et al. do teach the exact assay recited in Claim 133. Therefore, absent an unexpected result it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method reasonably suggested by the combination of Umansky et al. in view of Kiessling wherein the method of Chen et al. is used in place of the assay(s) reasonably suggested by the combination of Umansky et al. in view of Kiessling. Please note that substitution of one well known

method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

Umansky et al., Kiessling and/or Chen et al. teach the limitations recited in **Claims 134-142**.

Claim 206 is drawn to an embodiment of the method of Claim 133 wherein said agent is a cell lysis inhibitor.

Kiessling teach this limitation.

15. Claim(s) 143, 145-146 is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Umansky et al. [US 2002/0119478(2002)] in view of Saiki et al. [NEJM 46(2) : 301-302 (2000)] as applied against Claim 1 above and further in view of Livak et al. [US 5,538,848 (1996)].

Claim 143 is drawn to an embodiment of the method of Claim 1 wherein the DNA sample is analyzed using a particular assay.

Umansky et al. in view of Saiki et al. reasonably suggest a method of detecting the presence or absence of a fetal chromosomal abnormality which comprises all of the limitations recited in Claim 143 except these authors do not teach the particular assay recited therein. However, Livak et al. do teach the exact assay recited in Claims 143. Therefore, absent an unexpected result, it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method reasonably suggested

by the combination of Umansky et al. in view of Saiki et al. wherein the assay of Livak et al.. is used in place of the assay reasonably suggested by the combination of Umansky et al. in view of Saiki et al. Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

Livak et al. teach the limitations recited in **Claims 145-146**.

16. Claim(s) 144-146 and 206 is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Umansky et al. [US 2002/0119478(2002)] in view of Kiessling [US 5,618,664(1998)] as applied against Claim 58 above and further in view of Livak et al. [US 5,538,848 (1996)].

Claim 144 is drawn to an embodiment of the method of Claim 58 wherein the DNA sample is analyzed using a particular assay.

Umansky et al. in view of Kiessling reasonably suggest a method of detecting the presence or absence of a fetal chromosomal abnormality which comprises all of the limitations recited in Claim 144 except these authors do not teach the particular assay recited therein. However, Livak et al. do teach the exact assay recited in Claims 144. Therefore, absent an unexpected result, it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method reasonably suggested by the combination of Umansky et al. in view of Saiki et al. wherein the assay of Livak et

al.. is used in place of the assay reasonably suggested by the combination of Umansky et al. in view of Saiki et al. Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

Livak et al. teach the limitations recited in **Claims 145-146**.

Claim 206 is drawn to an embodiment of the method of Claim 144 wherein said agent is a cell lysis inhibitor.

Kiessling teach this limitation.

17. Claim(s) 148-151 is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Umansky et al. [US 2002/0119478(2002)] in view of Saiki et al. [NEJM 46(2) : 301-302 (2000)] and Chen et al. [Genome Research 10: 549-557(2000)]as applied against Claim 132 above and further in view of Kiessling [US 5,618,664(1998)].

Claim 148 is drawn to an embodiment of the method of Claim 132 wherein an agent that inhibits cell lysis has been added to the sample to inhibit the lysis of cells, if present , and wherein said agent is selected from a defined group which includes a cell lysis inhibitor. **Claim 149** is drawn to an embodiment of the method of Claim 148 wherein said agent is a cell lysis inhibitor.

Umansky et al. in view of Saiki et al. and Chen et al. reasonably suggest a method for detecting the presence or absence of a fetal chromosomal abnormality comprising all of the limitations recited in Claim 148 except these authors do not teach adding a cell lysis inhibitor to their sample . However, Kiessling do teach adding an agent to a biological sample which simultaneously disinfects said sample and fixes (i.e. inhibits the cell lysis of) WBCs therein. Therefore, absent an unexpected, it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to modify the method reasonably suggested by the combination of Umansky et al. in view of Saiki et al. and Chen et al. wherein an agent which inhibits cell lysis is mixed with the plasma sample prior to its analysis. The ordinary artisan would have been motivated to make the modification recited above in order to disinfect the plasma sample for the protection of the laboratory personnel handling and processing the plasma sample.

Claims 150-151 are drawn to embodiments of the method of Claim 149 wherein said cell lysis inhibitor is formalin at a particular concentration.

Kiessling teach this limitations, See Column 5, lines 36-56 of Kiessling wherein Kiessling teaches :

> "As used herein, the term "fixative" refers to an agent that is capable of preserving the structure of a biological molecule. Fixatives that are useful for the purposes of the instant invention include the well-known fixatives that are commonly used for flow cytometry and tissue fixation applications. (See, e.g., Lifson, J., et al., J. Immunol., Methods 86:143-149 (1986), the entire contents of which are incorporated by reference). These include, for example, paraformaldehyde, **formaldehyde and glutaraldehyde**. It is believed that fixatives, such as paraformaldehyde, act by crosslinking proteins, with the resulting crosslinked products stabilizing the cellular ultrastructure. (see, e.g., Aloisio, C., and Nicholson, J., J. Immunol. Methods. 128:281-285 (1990), the entire contents of which are incorporated by reference, and references cited therein). Also provided herein is a method for evaluating (screening) aldehyde and non-aldehyde (e.g., Streck's Tissue Fixative ("STF")) fixatives for use in accordance with the methods of the invention. (See e.g.,Science 260:976-979 (1993), the entire contents of which are incorporated herein by reference)."

Also note in Kiessling , the paragraph bridging Columns 5-6 where Kiessling teaches

> "In the preferred embodiments, the fixative solution contains between about 2% and about 10% paraformaldehyde. However, the preferred "disinfecting concentration" for a particular fixative is prescribed, at least in part, by

> the nature of the biological fluid. Thus, for blood samples in which it is desirable to simultaneously disinfect the sample, lyse the red blood cells and preserve the leukocytes for analysis, the preferred fixative is paraformaldehyde aving a disinfecting concentration of between about 2% and about 4%. This is because it is generally believed that paraformaldehyde concentrations less than 2% are not immediately (i.e., within 5-10 minutes) disinfecting, while concentrations greater than 4% fix (not lyse) the red blood cells."

It should be noted that formalin is simply a dilute aqueous solution of formaldehyde.

As regards the particular formalin concentrations recited in **Claims 150 and 151,** the applicant should note that where the general conditions of a claim are disclosed in the prior art, it is not inventive, in the absence of an unexpected result, to discover the optimum or workable ranges by routine experimentation. *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955).

18. Claim(s) 148-151 is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Umansky et al. [US 2002/0119478(2002)] in view of Saiki et al. [NEJM 46(2) : 301-302 (2000)] as applied against Claim 143 above and further in view of Livak et al. [US 5,538,848 (1996)] as applied against Claim 143 above and further in view of Kiessling [US 5,618,664(1998)].

Claim 148 is drawn to an embodiment of the method of Claim 132 wherein an agent that inhibits cell lysis has been added to the sample to inhibit the lysis of cells, if present , and wherein said agent is selected from a defined group which includes a cell lysis inhibitor. **Claim 149** is drawn to an embodiment of the method of Claim 148 wherein said agent is a cell lysis inhibitor.

Umansky et al. in view of Saiki et al. and Livak et al. reasonably suggest a method for detecting the presence or absence of a fetal chromosomal abnormality comprising all of the limitations recited in Claim 148 except these authors do not teach adding a cell lysis inhibitor to their sample . However, Kiessling do teach adding an agent to a biological sample which simultaneously disinfects said sample and fixes (i.e. inhibits the cell lysis of) WBCs therein. Therefore, absent an unexpected, it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to modify the method reasonably suggested by the combination of Umansky et al. in view of Saiki et al. and Livak et al. wherein an agent which inhibits cell lysis is mixed with the plasma sample prior to its analysis. The ordinary artisan would have been motivated to make the modification recited above in order to disinfect the plasma sample for the protection of the laboratory personnel handling and processing the plasma sample.

Claims 150-151 are drawn to embodiments of the method of Claim 149 wherein said cell lysis inhibitor is formalin at a particular concentration.

Kiessling teach this limitations, See Column 5, lines 36-56 of Kiessling wherein Kiessling teaches :

> "As used herein, the term "fixative" refers to an agent that is capable of preserving the structure of a biological molecule. Fixatives that are useful for the purposes of the instant invention include the well-known fixatives that are commonly used for flow cytometry and tissue fixation applications. (See, e.g., Lifson, J., et al., J. Immunol., Methods 86:143-149 (1986), the entire contents of which are incorporated by reference). These include, for example, paraformaldehyde, **formaldehyde and glutaraldehyde**. It is believed that fixatives, such as paraformaldehyde, act by crosslinking proteins, with the resulting crosslinked products stabilizing the cellular ultrastructure. (see, e.g., Aloisio, C., and Nicholson, J., J. Immunol. Methods. 128:281-285 (1990), the entire contents of which are incorporated by reference, and references cited therein). Also provided herein is a method for evaluating (screening) aldehyde and non-aldehyde (e.g., Streck's Tissue Fixative ("STF")) fixatives for use in accordance with the methods of the invention. (See e.g.,Science 260:976-979 (1993), the entire contents of which are incorporated herein by reference)."

Also note in Kiessling , the paragraph bridging Columns 5-6 where Kiessling teaches

> "In the preferred embodiments, the fixative solution contains between about 2% and about 10% paraformaldehyde. However, the preferred "disinfecting concentration" for a particular fixative is prescribed, at least in part, by

> the nature of the biological fluid. Thus, for blood samples in which it is desirable to simultaneously disinfect the sample, lyse the red blood cells and preserve the leukocytes for analysis, the preferred fixative is paraformaldehyde aving a disinfecting concentration of between about 2% and about 4%. This is because it is generally believed that paraformaldehyde concentrations less than 2% are not immediately (i.e., within 5-10 minutes) disinfecting, while concentrations greater than 4% fix (not lyse) the red blood cells."

It should be noted that formalin is simply a dilute aqueous solution of formaldehyde.

As regards the particular formalin concentrations recited in **Claims 150 and 151,** the applicant should note that where the general conditions of a claim are disclosed in the prior art, it is not inventive, in the absence of an unexpected result, to discover the optimum or workable ranges by routine experimentation. *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955).

19. Claim(s) 1-4, 8, 52, 56-57, 152 , 181-183, 185-187, 202-203, 205 and 207-208 is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Amicucci et al. [Clinical Chemistry 46(2) :301-302 (2000)] in view of Saiki et al. [NEJM 319 : 537-541 (1988)] .

Claim 1 is drawn to a method for detecting the presence or absence of a fetal chromosomal abnormality which method comprises quantitating the relative amount of the alleles at a heterozygous locus of interest wherein said heterozygous locus of interest has been determined by determining the sequence of the alleles at a locus of interest from template DNA obtained from a pregnant female, wherein said relative amount is expressed as a ratio, wherein said ratio indicates the presence or absence of a fetal chromosomal abnormality, and wherein said template DNA comprises a mixture of maternal DNA and fetal DNA.

Amicucci et al. teach a method of detecting the presence or absence of a fetal chromosomal abnormality which method comprises all of the limitations recited in Claim 1 except these authors do not teach expressing the relative amounts of the alleles as a ratio.

However, Saiki et al. do teach a method diagnosing sickle cell anemia wherein the relative amount of the alleles at a heterozygous locus of interest are determined and results of the assay are expressed as a ratio (i.e. $\beta^a \beta^a$ or $\beta^a \beta^s$ or $\beta^s \beta^s$). In light of these teachings and absent an unexpected result it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method of Umansky et al. wherein the results of the assay are expressed as a ratio as taught by Saiki et al. The ordinary artisan would have been motivated to make the modification of Umansky in order to quickly and succinctly relay the results of their prenatal tests to individual reading the results of said test(s). As regards the limitation which reads wherein said template DNA comprises a mixture of maternal DNA and fetal DNA. This limitation is inherent to Umansky in that both maternal and fetal free DNA present in the maternal blood stream will pass over into the maternal urine (i.e. maternal urine comprises both free fetal DNA as well as free maternal DNA).

Amicucci et al. or Saiki et al. teach the limitations recited in **Claims 2-4, 8, 52, and 56-57.**

Claim 152 is drawn to a method for detecting the presence or absence of a fetal chromosomal abnormality which method comprises two steps, To begin, the sequence of alleles of a locus of interest from template DNA is determined wherein the template DNA comprises a mixture of maternal DNA and fetal DNA and wherein said mixture is obtained from a pregnant female. Finally, the relative amount of the alleles at a heterozygous locus of interest that was identified from the locus of interest of step (a) is quantitated wherein the relative amount is expressed as a ratio and wherein the said ratio indicates the presence or absence of a fetal chromosomal abnormality

Amicucci et al. teach a method of detecting the presence or absence of a fetal chromosomal abnormality which method comprises all of the limitations recited in Claim 152 except these authors do not teach expressing the relative amounts of the alleles as a ratio. However, Saiki et al. do teach a method of diagnosing sickle cell anemia wherein the relative amount of the alleles at a heterozygous locus of interest are determined and results of the assay are expressed as a ratio (i.e. β^a β^a or β^a β^s or β^s β^s). In light of these teachings and absent an unexpected result it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method of Amicucci et al. wherein the results of the assay are expressed as a ratio as taught by Saiki et al. The ordinary artisan would have been motivated to make the modification of Amicucci et al. in order to quickly and succinctly relay the results of their prenatal tests to individual reading the results of said test(s). As regards the limitation which reads wherein said template DNA comprises a mixture of maternal DNA and fetal DNA. This limitation is inherent to Amicucci in that both maternal and fetal free DNA are present in maternal plasma. In support of this position please note the teaching present in Umansky et al. [US 2002/0119478(2002)]

Claim 181 is drawn to an embodiment of the method of Claim 1 wherein the sample is selected from a defined group that includes blood and plasma.

Claim 182 is drawn to an embodiment of the method of Claim 181 wherein the sample is blood. **Claim 183** is drawn to an embodiment of the method of Claim 182 wherein the template DNA is obtained from plasma from said blood. **Claim 185** is drawn to an embodiment of the method of Claim 8 wherein the sample is selected from a defined group that includes blood and plasma. **Claim 186** is drawn to an embodiment of the method of Claim 181 wherein the sample is blood. **Claim 187** is drawn to an embodiment of the method of Claim 182 wherein the template DNA is obtained from plasma from said blood. **Claim 202** is drawn to an embodiment of the method of Claim 152 wherein the sample is selected from a defined group that includes blood and plasma. **Claim 203** is drawn to an embodiment of the method of Claim 202 wherein the sample is blood. **Claim 205** is drawn to an embodiment of the method of Claim 203 wherein the template DNA is obtained from plasma from said blood.

Amicucci et al. teach these limitations in the 3rd paragraph wherein these authors teach isolating blood from the pregnant female which is then centrifuged and plasma isolated therefrom. These authors then teach isolating fetal DNA from the plasma.

Claim 207 is drawn to an embodiment of the method of Claim 1 or 152 wherein said mixture comprises at least about 15% fetal DNA. **Claim 208** is drawn to an embodiment of the method of Claim 1 or 152 wherein said mixture comprises a maximum of about 98-99%.

Claims 207-208 are very broad and encompass any percentage of fetal DNA present in said mixture. As evidenced by the teachings of Amicucci et al. the DNA extracted from the plasma of a pregnant female will comprise some percentage of fetal free DNA.

20. Claim(s) 184, 188 and 204 is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Amicucci et al. [Clinical Chemistry 46(2) :301-302 (2000)] in view of Saiki et al. [NEJM 319 : 537-541 (1988)] as applied against Claims 182, 186 and 203 above and further in view of Lo et al. {The Lancet 350 : 485-487 (1997)].

Claim 184 is drawn to an embodiment of the method of Claim 182 wherein the template DNA is obtained from serum from said blood. **Claim 188** is drawn to an embodiment of the method of Claim 186 wherein the template DNA is obtained from serum from said blood. **Claim 204** is drawn to an embodiment of the method of Claim 203 wherein the template DNA is obtained from serum from said blood.

Amicucci et al. in view of Saiki et al. reasonably suggest the methods recited in Claims 184, 188 and 204 except these authors do not teach isolating template DNA from blood serum, rather these authors teach isolating template DNA from blood plasma. However, as evidenced by at least Lo et al. the isolation and analysis of fetal template DNA from maternal blood serum was well known at the time of the invention. Therefore, absent an unexpected result it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method reasonably suggested by the combination of Amicucci et al. in view of Saiki et al. wherein maternal serum is used in place of maternal plasma. Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

21. Claim(s) 5-6, 20-24, 26-30, 32-34, 36-39, 41 and 44-52 and 56-57 is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Amicucci et al. [Clinical Chemistry 46(2) :301-302 (2000)] in view of Saiki et al. [NEJM 319 : 537-541 (1988)] as applied against Claim 1 above and further in view of Jones et al. [US 2003/0082576 (2003)].

Claim 5 is drawn to an embodiment of the method of Claim 1 wherein alleles of multiple loci of interest are sequenced and their relative amounts are expressed as a ratio.

Amicucci et al. in view of Saiki et al. reasonably suggest a method of detecting the presence or absence of a fetal chromosomal abnormality comprising all of the limitations recited in Claim 5 except these authors do not teach sequencing multiple loci of interest. However, as evidenced by at least Jones et al. such multiplexing was routine in the art at the time of the invention. In light of these findings, and absent an unexpected, it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to modify the method reasonably suggested by the combination of Amicucci et al. in view of Saiki et al. wherein multiple loci on multiple chromosomes are analyzed. The ordinary artisan would have been motivated to make the modification recited above in order to analyze multiple different disease/disorder associated genes for mutations simultaneously (i.e. in a single assay).

Amicucci et al. ,Saiki et al. or Jones et al. teach the limitations recited in **Claims 6, 20-24, 26-30, 32-34, 36-39, 41 and 44-52 and 56-57.**

22. **Claim(s) 9-12, 14-15, 18-19 and 189** is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Amicucci et al. [Clinical Chemistry 46(2) :301-302 (2000)] in view of Saiki et al. [NEJM 319 : 537-541 (1988)] as applied against Claim 4 above and further in view of Kiessling [US 5,618,664(1998)].

Claim 9 is drawn to an embodiment of the method of Claim 4 wherein the sample is mixed with an agent that inhibits cell lysis wherein the agent is selected from a defined group which includes a cell lysis inhibitor.

Amicucci et al. in view of Saiki et al. reasonably suggest a method of detecting the presence or absence of a fetal chromosomal abnormality comprising all of the limitations recited in Claim 9 except these authors do not teach mixing their sample with an agent that inhibits cell lysis wherein the agent is selected from a defined group which includes a cell lysis inhibitor. However as evidenced by Kiessling the addition of an agent to a biological sample which simultaneously disinfects and inhibits the lysis of cells therein (inhibits the lysis of WBCs) was well known prior to the instant invention. Therefore, absent an unexpected, it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to modify the method reasonably suggested by the combination of Amicucci et al. in view of Saiki et al. wherein an agent which inhibits cell lysis is mixed with the serum sample prior to its analysis. The ordinary artisan would have been motivated to make the modification recited above in order to disinfect the plasma sample of Amicucci et al. in view of Saiki et al. for the protection of the laboratory personnel performing the assay.

Amicucci et al., Saiki et al. or Kiessling teach the limitations recited in **Claims 10-11.**

Claim 12 is drawn to an embodiment of the method of Claim 9 wherein

the sample is blood. **Claim 14** is drawn to an embodiment of the method of Claim 12 wherein said blood is obtained from a pregnant female sometime during her pregnancy. **Claim 15** is drawn to an embodiment of the method of Claim 12 wherein the template DNA is obtained from plasma from said blood.

Amicucci et al. teach these limitations in the 3rd paragraph wherein these authors teach isolating blood from the pregnant female which is then centrifuged and plasma isolated therefrom. These authors then teach isolating fetal DNA from the plasma.

Claim 18 is drawn to an embodiment of the method of Claim 15 or 16 wherein prior to determining the sequence of the locus of interest on fetal DNA the sequence of the locus of interest on maternal template DNA is determined. **Claim 19** is drawn to an embodiment of the method of Claim 15 or 16 wherein prior to determining the sequence of the locus of interest on fetal DNA the sequence of the locus of interest on maternal (? paternal) template DNA is determined.

As regards the limitations present in Claims 18-19, it must be noted that none of Amicucci et al. or Saiki et al. teach testing the maternal and/or paternal DNA prior to testing the fetal DNA. Saiki et al. do teach testing both maternal and paternal DNA just not prior to testing the fetal DNA. However, absent an unexpected result it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to modify the method reasonably suggested by the combination of Umansky et al. in view of Saiki et al. wherein maternal and/or paternal DNA is tested prior to or simultaneously with the fetal DNA. It would have been *prima facie* obvious to an ordinary practitioner to switch the order of ingredient addition see MPEP 2144.04 which refers to *In re Gibson*, 39 F.2d 975,5 USPQ 230 (CCPA 1930). Selection of any order of mixing ingredients is *prima facie* obvious".

Claim 189 is drawn to an embodiment of the method of Claim 11 wherein said cell lysis inhibitor is selected from a defined group which includes gluteraldehyde, formaldehyde and formalin.

Kiessling teach these limitations, See Column 5, lines 36-56 of Kiessling wherein Kiessling teaches :

> "As used herein, the term "fixative" refers to an agent that is capable of preserving the structure of a biological molecule. Fixatives that are useful for the purposes of the instant invention include the well-known fixatives that are commonly used for flow cytometry and tissue fixation applications. (See, e.g., Lifson, J., et al., J. Immunol., Methods 86:143-149 (1986), the entire contents of which are incorporated by reference). These include, for example, paraformaldehyde, **formaldehyde and glutaraldehyde**. It is believed that fixatives, such as paraformaldehyde, act by crosslinking proteins, with the resulting crosslinked products stabilizing the cellular ultrastructure. (see, e.g., Aloisio, C., and Nicholson, J., J. Immunol. Methods. 128:281-285 (1990), the entire contents of which are incorporated by reference, and references cited therein). Also provided herein is a method for evaluating (screening) aldehyde and non-aldehyde (e.g., Streck's Tissue Fixative ("STF")) fixatives for use in accordance with the methods of the invention. (See e.g.,Science 260:976-979 (1993), the entire contents of which are incorporated herein by reference)."

Also note in Kiessling , the paragraph bridging Columns 5-6 where Kiessling teaches

> "In the preferred embodiments, the fixative solution contains between about 2% and about 10% paraformaldehyde. However, the preferred "disinfecting concentration" for a particular fixative is prescribed, at least in part, by the nature of the biological fluid. Thus, for blood samples in which it is desirable to simultaneously disinfect the sample, lyse the red blood cells and preserve the leukocytes for analysis, the preferred fixative is paraformaldehyde aving a disinfecting concentration of between about 2% and about 4%. This is because it is generally believed that paraformaldehyde concentrations less than 2% are not immediately (i.e., within 5-10 minutes) disinfecting, while concentrations greater than 4% fix (not lyse) the red blood cells."

It should be noted that formalin is simply a dilute aqueous solution of formaldehyde.

23. Claim(s) 16 and 18-19 is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Amicucci et al. [Clinical Chemistry 46(2) :301-302 (2000)] in view of Saiki et al. [NEJM 319 : 537-541 (1988)] and Kiessling [US 5,618,664(1998)] as applied against Claim 12 above and further in view of Lo et al. [The Lancet 350: 485-487 (1997)].

Claim 16 is drawn to an embodiment of the method of Claim 12 wherein the template DNA is obtained from serum from said blood.

Amicucci et al. in view of Saiki et al. and Kiessling reasonably suggest the methods recited in Claims 16 except these authors do not teach isolating template DNA from blood serum, rather these authors teach isolating template DNA from blood plasma. However , as evidenced by at least Lo et al. the isolation and analysis of fetal template DNA from maternal blood serum was well known at the time of the invention. Therefore, absent an unexpected result it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method reasonably suggested by the combination of Amicucci et al. in view of Saiki et al. wherein the maternal serum is used in place of maternal plasma. Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

Claim 18 is drawn to an embodiment of the method of Claim 15 or 16 wherein prior to determining the sequence of the locus of interest on fetal DNA the sequence of the locus of interest on maternal template DNA is determined. **Claim 19** is

drawn to an embodiment of the method of Claim 15 or 16 wherein prior to determining the sequence of the locus of interest on fetal DNA the sequence of the locus of interest on maternal (? paternal) template DNA is determined.

As regards the limitations present in Claims 18-19, it must be noted that none of Amicucci et al., Saiki et al. or Lo et al. teach testing the maternal and/or paternal DNA prior to testing the fetal DNA. Saiki et al. do teach testing both maternal and paternal DNA just not prior to testing the fetal DNA. However, absent an unexpected result it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to modify the method reasonably suggested by the combination of Umansky et al. in view of Saiki et al. wherein maternal and/or paternal DNA is tested prior to or simultaneously with the fetal DNA. It would have been *prima facie* obvious to an ordinary practitioner to switch the order of ingredient addition see MPEP 2144.04 which refers to *In re Gibson*, 39 F.2d 975,5 USPQ 230 (CCPA 1930). Selection of any order of mixing ingredients is *prima facie* obvious".

24. **Claim(s) 43** is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Amicucci et al. [Clinical Chemistry 46(2) :301-302 (2000)] in view of Saiki et al. [NEJM 319 : 537-541 (1988)] and Jones et al. [US 2003/0082576 (2003)] as applied against Claim 20 or 21 above and further in view of MacLeod et al. [US 6,221,600 (2001)] and Polisson [US 5,098,839 (1992)].

Claim 43 is drawn to an embodiment of the method of Claim 1 wherein the restriction enzyme recognition site is for a restriction enzyme selected from a defined group which includes : BsaJ I and Bssk I and Dde I and EcoNI and Fnu4H I and Hinf I and ScrF I.

Amicucci et al. in view of Saiki et al. and Jones et al. reasonably suggest a method comprising all of the limitations of Claim 43 except these authors do not teach the restriction enzymes recited. However, as evidenced by MacLeod et al. and Polisson the restriction enzymes recited well known prior to the instant invention. Therefore, absent an unexpected result, it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method reasonably suggested by the combination of Amicucci et al. in view of Saiki et al. and Jones et al. wherein the Type IIS restriction enzymes taught by MacLeod et al. and Polisson are used rather the Type IIS restriction enzymes taught in Jones et al. Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

25. Claim(s) 58-65, 67-68, 87-102, 190-194, 196, and 201 is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Amicucci et al. [Clinical Chemistry 46(2) :301-302 (2000)] in view of Kiessling [US 5,618,664(1998)].

Claim 58 is drawn to a method which comprises determining the sequence of a locus of interest on free fetal DNA from a sample comprising free fetal DNA wherein an agent that inhibits cell lysis has been added to said sample to inhibit the lysis of cells present therein, wherein said agent is selected from a defined group which includes membrane stabilizer, cross-linker, and cell lysis inhibitor.

Amicucci et al. teach a method of determining the sequence of a fetal locus of interest (i.e. diagnosing Myotonic dystrophy from a maternal blood plasma sample which utilizes free fetal DNA present in the maternal plasma. In summary, Amicucci et al. teach a method comprising all of the limitations of Claim 58 except these authors do not teach adding an agent that inhibits cell lysis to their maternal samples. However as evidenced by Kiessling the addition of an agent to a biological sample which simultaneously disinfects and inhibits the lysis of cells therein (inhibits the lysis of WBCs) was well known prior to the instant invention. Therefore, absent an unexpected result, it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method of Amicucci et al. wherein the maternal sample is treated according to the method of Kiessling. The ordinary artisan would have been motivated to make the modification recited above in order to reduce the risk of viral infection for those laboratory personnel performing the assay of Amicucci et al. It is noted that the fixative(s) taught by Kiessling lyses RBCs while fixing (i.e. inhibiting the lysis of) WBCs.

Claim 59 is drawn to an embodiment of the method of Claim 58 wherein the sample is selected from a defined group which includes blood and plasma. **Claim 60** is drawn to an embodiment of the method of Claim 59 wherein the sample is blood. **Claim 65** is drawn to an embodiment of the method of Claim 60 wherein said template DNA is obtained from plasma of said blood.

Amicucci et al. teach these limitations in the 3rd paragraph wherein these authors teach isolating blood from the pregnant female which is then centrifuged and plasma isolated therefrom. These authors then teach isolating fetal DNA from the plasma.

Claim 61 is drawn to an embodiment of the method of Claim 58 wherein the sample comprises free maternal template DNA and free fetal template DNA.

Admittedly, none of Amicucci et al. or Kiessling teach this limitation. However, this limitation is considered inherent to Amicucci et al. in that the blood of a pregnant

female will normally comprises both maternal free DNA and fetal free DNA which results from the leakage of cellular DNA into the maternal blood following cell death. In support of this position note the teachings in Umansky et al. [US 2002/0119478 (2002)].

Amicucci et al. or Kiessling teach the limitations recited in **Claims 59, 62-64, and 190-192.**

Claim 67 is drawn to an embodiment of the method of Claim 58 wherein prior to determining the sequence of the locus of interest on fetal DNA the sequence of the locus of interest on maternal template DNA is determined. **Claim 68** is drawn to an embodiment of the method of Claim 58 wherein prior to determining the sequence of the locus of interest on fetal DNA the sequence of the locus of interest on paternal template DNA is determined.

As regards the limitations present in Claims 67-68, it must be noted (Official Notice) that while none of Amicucci et al. or Kiessling teach testing the maternal and/or paternal DNA prior to testing the fetal DNA, it would have been, absent an unexpected result, *prima facie* obvious to one of ordinary skill in the art at the time of the invention to modify the method reasonably suggested by the combination of Amicucci et al. in view of Kiessling wherein maternal and/or paternal DNA is tested prior to, or simultaneously with the fetal DNA.

Claim 87 is drawn to a method for preparing a sample for analysis comprising isolating free nucleic acid from a sample that contains nucleic acid wherein an agent that inhibits cell lysis is added to the sample to inhibit the lysis of cells, if cells are present wherein the agent is selected from a defined group which includes a cell lysis inhibitor.

Amicucci et al. teach a method for preparing a sample for analysis which method comprises all of the limitations recited in Claim 87 except these authors do not teach adding an agent that inhibits cell lysis is added to the sample to inhibit the lysis of cells, if cells are present wherein the agent is selected from a defined group which includes a cell lysis inhibitor. However as evidenced by Kiessling the addition of an agent to a biological sample which simultaneously disinfects and inhibits the lysis of cells therein. Therefore, absent an unexpected result, it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method of Amicucci et al. wherein the maternal sample is treated according to the method of Kiessling. The ordinary artisan would have been motivated to make the modification recited above in order to reduce the risk of viral infection for those laboratory personnel performing the assay of Amicucci et al. It is noted that the fixative(s) taught by Kiessling lyses RBCs while fixing (i.e. inhibiting the lysis of) WBCs.

Amicucci et al. and/or Kiessling teach the limitations recited in **Claims 88-89.**

Claim 90 is drawn to an embodiment of the method of Claim 87 wherein the sample is selected from a defined group which includes blood and plasma. **Claim 91** is drawn to an embodiment of the method of Claim 90 wherein the sample is blood. **Claim 92** is drawn to an embodiment of the method of Claim 91 wherein said blood is from a pregnant female. **Claim 93** is drawn to an embodiment of the method of Claim 92 wherein said blood is obtained from a pregnant female some time during her pregnancy **Claim 94** is drawn to an embodiment of the method of Claim 60 wherein said template DNA is obtained from plasma of said blood.

Amicucci et al. teach these limitations in the 3rd paragraph wherein these authors teach isolating blood from the pregnant female which is then centrifuged and plasma isolated therefrom. These authors then teach isolating fetal DNA from the plasma.

Amicucci et al. and/or Kiessling teach the limitations recited in **Claims 95-96.**

Claim 97 is drawn to an embodiment of the method of Claim 87 wherein said cell lysis inhibitor is formalin.

Kiessling teach this limitations, See Column 5, lines 36-56 of Kiessling wherein Kiessling teaches :

> "As used herein, the term "fixative" refers to an agent that is capable of preserving the structure of a biological molecule. Fixatives that are useful for the purposes of the instant invention include the well-known fixatives that are commonly used for flow cytometry and tissue fixation applications. (See, e.g., Lifson, J., et al., J. Immunol., Methods 86:143-149 (1986), the entire contents of which are incorporated by reference). These include, for example, paraformaldehyde, **formaldehyde and glutaraldehyde**. It is believed that fixatives, such as paraformaldehyde, act by crosslinking proteins, with the resulting crosslinked products stabilizing the cellular ultrastructure. (see, e.g., Aloisio, C., and Nicholson, J., J. Immunol. Methods. 128:281-285 (1990), the entire contents of which are incorporated by reference, and references cited therein). Also provided herein is a method for evaluating (screening) aldehyde and non-aldehyde (e.g., Streck's Tissue Fixative ("STF")) fixatives for use in accordance with the methods of the invention. (See e.g.,Science 260:976-979 (1993), the entire contents of which are incorporated herein by reference)."

Also note in Kiessling , the paragraph bridging Columns 5-6 where Kiessling teaches

> "In the preferred embodiments, the fixative solution contains between about 2% and about 10% paraformaldehyde. However, the preferred "disinfecting concentration" for a particular fixative is prescribed, at least in part, by the nature of the biological fluid. Thus, for blood samples in which it is desirable to simultaneously disinfect the sample, lyse the red blood cells and preserve the leukocytes for analysis, the preferred fixative is paraformaldehyde aving a disinfecting concentration of between about 2% and about 4%. This is because it is generally believed that paraformaldehyde concentrations less than 2% are not immediately (i.e., within 5-10 minutes) disinfecting, while concentrations greater than 4% fix (not lyse) the red blood cells."

It should be noted that formalin is simply a dilute aqueous solution of formaldehyde.

As regards the particular formalin concentrations recited in **Claims 98-99** The applicant should note that where the general conditions of a claim are disclosed in the prior art, it is not inventive, in the absence of an unexpected result, to discover the

optimum or workable ranges by routine experimentation. *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233,.235 (CCPA 1955).

Amicucci et al. and/or Kiessling teach the limitation recited in **Claim 100 and 102.**

Claim 101 is drawn to an embodiment of the method of Claim 100 wherein the centrifugation step is performed with the centrifuge braking power set to zero.

Admittedly , neither of Amicucci et al. or Kiessling teach this limitation. However, it must be noted (Official Notice) that it was routine in the art to perform centrifugation step(s) wherein the centrifuge braking power set to zero. This is often done in steps involving DNA/RNA extraction with organic solvents in order to prevent disruption of the interface between the organic phase and the aqueous phase.

Amicucci et al. and/or Kiessling teach the limitation recited in **Claims 190-192.**

Claim 193 is drawn to an embodiment of the method of Claim 192 wherein the sample is blood. **Claim 194** is drawn to an embodiment of the method of Claim 193 wherein said template DNA is obtained from plasma of said blood.

Amicucci et al. teach these limitations in the 3rd paragraph wherein these authors teach isolating blood from the pregnant female which is then centrifuged and plasma isolated therefrom. These authors then teach isolating fetal DNA from the plasma.

Claim 196 is drawn to an embodiment of the method of Claim 63 wherein said cell lysis inhibitor is selected from a defined group which includes glutaraldehyde, formaldehyde and formalin. **Claim 201** is drawn to an embodiment of the method of Claim 96 wherein said cell lysis inhibitor is selected from a defined group which includes glutaraldehyde, formaldehyde and formalin.

Kiessling teach these limitations, See Column 5, lines 36-56 of Kiessling wherein Kiessling teaches :

> "As used herein, the term "fixative" refers to an agent that is capable of preserving the structure of a biological molecule. Fixatives that are useful for the purposes of the instant invention include the well-known fixatives that are commonly used for flow cytometry and tissue fixation applications. (See, e.g., Lifson, J., et al., J. Immunol., Methods 86:143-149 (1986), the entire contents of which are incorporated by reference). These include, for example, paraformaldehyde, **formaldehyde and glutaraldehyde**. It is believed that fixatives, such as paraformaldehyde, act by crosslinking proteins, with the resulting crosslinked products stabilizing the cellular ultrastructure. (see, e.g., Aloisio, C., and Nicholson, J., J. Immunol. Methods. 128:281-285 (1990), the entire contents of which are incorporated by reference, and references cited therein). Also provided herein is a method for evaluating (screening) aldehyde and non-aldehyde (e.g., Streck's Tissue Fixative ("STF")) fixatives for use in accordance with the methods of the invention. (See e.g.,Science 260:976-979 (1993), the entire contents of which are incorporated herein by reference)."

Also note in Kiessling , the paragraph bridging Columns 5-6 where Kiessling teaches

> "In the preferred embodiments, the fixative solution contains between about 2% and about 10% paraformaldehyde. However, the preferred "disinfecting concentration" for a particular fixative is prescribed, at least in part, by the nature of the biological fluid. Thus, for blood samples in which it is desirable to simultaneously disinfect the sample, lyse the red blood cells and preserve the leukocytes for analysis, the preferred fixative is paraformaldehyde aving a disinfecting concentration of between about 2% and about 4%. This is because it is generally believed that paraformaldehyde concentrations less than 2% are not immediately (i.e., within 5-10 minutes) disinfecting, while concentrations greater than 4% fix (not lyse) the red blood cells."

It should be noted that formalin is simply a dilute aqueous solution of formaldehyde.

26. Claim(s) 66 and 195 is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Amicucci et al. [Clinical Chemistry 46(2) :301-302 (2000)] in view of Kiessling [US 5,618,664(1998)] as applied against Claim 60 above and further in view of Lo et al. [The Lancet 350: 485-487 (1997)].

Claim 66 is drawn to an embodiment of the method of Claim 60 wherein the template DNA is obtained from serum from said blood. **Claim 195** is drawn to an embodiment of the method of Claim 193 wherein the template DNA is obtained from serum from said blood.

Amicucci et al. in view of Kiessling reasonably suggest the method recited in Claims 65 and 195 except these authors do not teach isolating template DNA from blood serum, rather these authors teach isolating template DNA from blood plasma. However , as evidenced by at least Lo et al. the isolation and analysis of fetal template DNA from maternal blood serum was well known at the time of the invention. Therefore, absent an unexpected result it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method reasonably suggested by the combination of Amicucci et al. in view of Saiki et al. wherein the maternal serum is used in place of maternal plasma. Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

27. Claim(s) 69-70 and 83 is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Amicucci et al. [Clinical Chemistry 46(2) :301-302 (2000)] in view of Kiessling [US 5,618,664(1998)] as applied against Claim 58 above and further in view of Saiki et al. [NEJM 319 : 537-541 (1988)].

Claim 69 is drawn to an embodiment of the method of Claim 58 wherein said locus of interest is a SNP. **Claim 70** is drawn to an embodiment of the method of Claim 58 wherein said locus of interest is a mutation.

Amicucci et al. in view of Kiessling reasonably suggest a method comprising all of the limitations of Claim 69-70 except these authors do not teach an embodiment wherein the locus of interest is a SNP or a mutation. However, as evidenced by at least Saiki et al. the analysis of a the locus of interest that is a SNP or a mutation was well known prior to the instant invention. See at least for example the abstract. Therefore, absent an unexpected result, it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method reasonably suggested by the combination of Amicucci et al. in view of Kiessling wherein the locus analyzed is a SNP or a mutation. Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

Claim 83 is drawn to an embodiment of the method of Claim 58 the sequence of said locus of interest is determined using a method selected from a defined group which includes dot blots.

Saiki et al. teach this limitation.

28. Claim(s) 71-83 is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Amicucci et al. [Clinical Chemistry 46(2) :301-302 (2000)] in view of Kiessling [US 5,618,664(1998)] as applied against Claim 58 above and further in view of Jones et al. [US 2003/0082576 (2003)]

Claim 71 is drawn to an embodiment of the method of Claim 58 wherein the sequence of multiple loci of interest is determined.

Amicucci et al. in view of Kiessling reasonably suggest a method of determining the sequence of a locus of interest on free fetal DNA comprising all of the limitations recited in Claim 71 except these authors do not teach sequencing multiple loci of interest. However, as evidenced by at least Jones et al. such multiplexing was routine in the art at the time of the invention. In light of these findings, and absent an unexpected, it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to modify the method reasonably suggested by the combination of Amicucci et al. in view of Kiessling wherein multiple loci on multiple chromosomes are analyzed. The ordinary artisan would have been motivated to make the modification recited above in order to analyze multiple different disease/disorder associated genes for mutations simultaneously (i.e. in a single assay).

Amicucci et al. , Kiessling or Jones et al. teach the limitations recited in **Claims 72-83.**

29. Claim(s) 132, and 134-142 is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Amicucci et al. [Clinical Chemistry 46(2) :301-302 (2000)] in view of Saiki et al. [NEJM 319 : 537-541 (1988)] as applied against Claim 1 above and further in view of Chen et al. [Genome Research 10: 549-557(2000)].

Claim 132 is drawn to an embodiment of the method of Claim 1 wherein said sequence is determined using a particular assay.

Amicucci et al. in view of Saiki et al. reasonably suggest a method for detecting the presence or absence of a fetal chromosomal abnormality comprising all of the limitations recited in Claim 132 except these authors do not teach the particular assay recited therein. However, Chen et al. do teach the exact assay recited in Claim 132. Therefore, absent an unexpected result it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method reasonably suggested by the combination of Amicucci et al. in view of Saiki et al. wherein the method of Chen et al. is used in place of the assay(s) reasonably suggested by the combination of Amicucci et al. in view of Saiki et al. Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

Amicucci et al. Saiki et al. or Chen et al. teach the limitations recited in **Claims 134-142.**

30. Claim(s) 133-142 and 206 is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Amicucci et al. [Clinical Chemistry 46(2) :301-302 (2000)] in view of Kiessling [US 5,618,664(1998)] as applied against Claim 58 above and further in view of Chen et al. [Genome Research 10: 549-557(2000)].

Claim 133 is drawn to an embodiment of the method of Claim 58 wherein said sequence is determined using a particular assay.

Amicucci et al. in view of Kiessling reasonably suggest a method for determining the sequence of a locus of interest on free fetal DNA comprising all of the limitations

recited in Claim 133 except these authors do not teach the particular assay recited in Claim 133. However, Chen et al. do teach the exact assay recited in Claim 133. Therefore, absent an unexpected result it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method reasonably suggested by the combination of Amicucci et al. in view of Kiessling wherein the method of Chen et al. is used in place of the assay(s) reasonably suggested by the combination of Amicucci et al. in view of Kiessling. Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

Amicucci et al., Kiessling or Chen et al. teach the limitations recited in **Claims 134-142 and 206.**

31. Claim(s) 143, 145-146 is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Amicucci et al. [Clinical Chemistry 46(2) :301-302 (2000)] in view of Saiki et al. [NEJM 319 : 537-541 (1988)] as applied against Claim 1 above and further in view of Livak et al. [US 5,538,848 (1996)].

Claim 143 is drawn to an embodiment of the method of Claim 1 wherein the DNA sample is analyzed using a particular assay.

Amicucci et al. in view of Saiki et al. reasonably suggest a method of detecting the presence or absence of a fetal chromosomal abnormality which comprises all of the limitations recited in Claim 143 except these authors do not teach the particular assay

recited therein. However, Livak et al. do teach the exact assay recited in Claims 143. Therefore, absent an unexpected result, it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method reasonably suggested by the combination of Amicucci et al. in view of Saiki et al. wherein the assay of Livak et al.. is used in place of the assay reasonably suggested by the combination of Amicucci et al. in view of Saiki et al. Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

Amicucci et al. , Saiki et al. or Livak et al. teach the limitations recited in **Claims 145-146.**

32. Claim(s) 144-146 and 206 is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Amicucci et al. [Clinical Chemistry 46(2) :301-302 (2000)] in view of Kiessling [US 5,618,664(1998)] as applied against Claim 58 above and further in view of Chen et al. [Genome Research 10: 549-557(2000)].

Claim 144 is drawn to an embodiment of the method of Claim 58 wherein said sequence is determined using a particular assay.

Amicucci et al. in view of Kiessling reasonably suggest a method for determining the sequence of a locus of interest on free fetal DNA comprising all of the limitations recited in Claim 144 except these authors do not teach the particular assay recited therein However, Livak et al. do teach the exact assay recited in Claim 144. Therefore,

absent an unexpected result it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method reasonably suggested by the combination of Amicucci et al. in view of Kiessling wherein the method of Chen et al. is used in place of the assay(s) reasonably suggested by the combination of Umansky et al. in view of Kiessling. Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

Amicucci et al., Kiessling or Livak et al. teach the limitations recited in **Claims 145-146 and 206.**

33. **Claim(s) 148-151** is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Amicucci et al. [Clinical Chemistry 46(2) :301-302 (2000)] in view of Saiki et al. [NEJM 319 : 537-541 (1988)] and Chen et al. [Genome Research 10: 549-557(2000)] as applied against Claim 132 above and further in view of Kiessling [US 5,618,664(1998)].

Claim 148 is drawn to an embodiment of the method of Claim 132 wherein an agent that inhibits cell lysis has been added to the sample to inhibit the lysis of cells, if present , and wherein said agent is selected from a defined group which includes a cell lysis inhibitor. **Claim 149** is drawn to an embodiment of the method of Claim 148 wherein said agent is a cell lysis inhibitor.

Amicucci et al. in view of Saiki et al. and Chen et al. reasonably suggest a method for detecting the presence or absence of a fetal chromosomal abnormality comprising all of the limitations recited in Claim 132 except these authors do not teach adding a cell lysis inhibitor to their sample . However, Kiessling do teach adding an agent to a biological sample which simultaneously disinfects said sample and fixes (i.e. inhibits the cell lysis of) WBCs therein. Therefore, absent an unexpected, it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to modify the method reasonably suggested by the combination of Amicucci et al. in view of Saiki et al. and Chen et al. wherein an agent which inhibits cell lysis is mixed with the plasma sample prior to its analysis. The ordinary artisan would have been motivated to make the modification recited above in order to disinfect the plasma sample for the protection of the laboratory personnel handling and processing the plasma sample.

Claims 150-151 are drawn to embodiments of the method of Claim 149 wherein said cell lysis inhibitor is formalin at a particular concentration.

Kiessling teach this limitations, See Column 5, lines 36-56 of Kiessling wherein Kiessling teaches :

> "As used herein, the term "fixative" refers to an agent that is capable of preserving the structure of a biological molecule. Fixatives that are useful for the purposes of the instant invention include the well-known fixatives that are commonly used for flow cytometry and tissue fixation applications. (See, e.g., Lifson, J., et al., J. Immunol., Methods 86:143-149 (1986), the entire contents of which are incorporated by reference). These include, for example, paraformaldehyde, **formaldehyde and glutaraldehyde**. It is believed that fixatives, such as paraformaldehyde, act by crosslinking proteins, with the resulting crosslinked products stabilizing the cellular ultrastructure. (see, e.g., Aloisio, C., and Nicholson, J., J. Immunol. Methods. 128:281-285 (1990), the entire contents of which are incorporated by reference, and references cited therein). Also provided herein is a method for evaluating (screening) aldehyde and non-aldehyde (e.g., Streck's Tissue Fixative ("STF")) fixatives for use in accordance with the methods of the invention. (See e.g.,Science 260:976-979 (1993), the entire contents of which are incorporated herein by reference)."

Also note in Kiessling , the paragraph bridging Columns 5-6 where Kiessling teaches

> "In the preferred embodiments, the fixative solution contains between about 2% and about 10% paraformaldehyde. However, the preferred "disinfecting concentration" for a particular fixative is prescribed, at least in part, by

> the nature of the biological fluid. Thus, for blood samples in which it is desirable to simultaneously disinfect the sample, lyse the red blood cells and preserve the leukocytes for analysis, the preferred fixative is paraformaldehyde aving a disinfecting concentration of between about 2% and about 4%. This is because it is generally believed that paraformaldehyde concentrations less than 2% are not immediately (i.e., within 5-10 minutes) disinfecting, while concentrations greater than 4% fix (not lyse) the red blood cells."

It should be noted that formalin is simply a dilute aqueous solution of formaldehyde.

As regards the particular formalin concentrations recited in **Claims 150 and 151,** the applicant should note that where the general conditions of a claim are disclosed in the prior art, it is not inventive, in the absence of an unexpected result, to discover the optimum or workable ranges by routine experimentation. *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955).

34. **Claim(s) 148-151** is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Amicucci et al. [Clinical Chemistry 46(2) :301-302 (2000)] in view of Saiki et al. [NEJM 319 : 537-541 (1988)] and Livak et al. [US 5,538,848 (1996)].
as applied against Claim 143 above and further in view of Kiessling [US 5,618,664(1998)].

Claim 148 is drawn to an embodiment of the method of Claim 143 wherein an agent that inhibits cell lysis has been added to the sample to inhibit the lysis of cells, if present , and wherein said agent is selected from a defined group which includes a cell lysis inhibitor. **Claim 149** is drawn to an embodiment of the method of Claim 148 wherein said agent is a cell lysis inhibitor.

Amicucci et al. in view of Saiki et al. and Livak et al. reasonably suggest a method for detecting the presence or absence of a fetal chromosomal abnormality comprising all of the limitations recited in Claim 148 except these authors do not teach adding a cell lysis inhibitor to their sample . However, Kiessling do teach adding an agent to a biological sample which simultaneously disinfects said sample and fixes (i.e. inhibits the cell lysis of) WBCs therein. Therefore, absent an unexpected, it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to modify the method reasonably suggested by the combination of Amicucci et al. in view of Saiki et al. and Livak et al. wherein an agent which inhibits cell lysis is mixed with the plasma sample prior to its analysis. The ordinary artisan would have been motivated to make the modification recited above in order to disinfect the plasma sample for the protection of the laboratory personnel handling and processing the plasma sample.

Claims 150-151 are drawn to embodiments of the method of Claim 149 wherein said cell lysis inhibitor is formalin at a particular concentration.

Kiessling teach this limitations, See Column 5, lines 36-56 of Kiessling wherein Kiessling teaches :

> "As used herein, the term "fixative" refers to an agent that is capable of preserving the structure of a biological molecule. Fixatives that are useful for the purposes of the instant invention include the well-known fixatives that are commonly used for flow cytometry and tissue fixation applications. (See, e.g., Lifson, J., et al., J. Immunol., Methods 86:143-149 (1986), the entire contents of which are incorporated by reference). These include, for example, paraformaldehyde, **formaldehyde and glutaraldehyde**. It is believed that fixatives, such as paraformaldehyde, act by crosslinking proteins, with the resulting crosslinked products stabilizing the cellular ultrastructure. (see, e.g., Aloisio, C., and Nicholson, J., J. Immunol. Methods. 128:281-285 (1990), the entire contents of which are incorporated by reference, and references cited therein). Also provided herein is a method for evaluating (screening) aldehyde and non-aldehyde (e.g., Streck's Tissue Fixative ("STF")) fixatives for use in accordance with the methods of the invention. (See e.g.,Science 260:976-979 (1993), the entire contents of which are incorporated herein by reference)."

Also note in Kiessling , the paragraph bridging Columns 5-6 where Kiessling teaches

> "In the preferred embodiments, the fixative solution contains between about 2% and about 10% paraformaldehyde. However, the preferred "disinfecting concentration" for a particular fixative is prescribed, at least in part, by

> the nature of the biological fluid. Thus, for blood samples in which it is desirable to simultaneously disinfect the sample, lyse the red blood cells and preserve the leukocytes for analysis, the preferred fixative is paraformaldehyde aving a disinfecting concentration of between about 2% and about 4%. This is because it is generally believed that paraformaldehyde concentrations less than 2% are not immediately (i.e., within 5-10 minutes) disinfecting, while concentrations greater than 4% fix (not lyse) the red blood cells."

It should be noted that formalin is simply a dilute aqueous solution of formaldehyde.

As regards the particular formalin concentrations recited in **Claims 150 and 151,** the applicant should note that where the general conditions of a claim are disclosed in the prior art, it is not inventive, in the absence of an unexpected result, to discover the optimum or workable ranges by routine experimentation. *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955).

35. Claim(s) 87-96 is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Kiessling [US 5,618,664(1998)].

Claim 87 is drawn to a method for preparing a sample for analysis comprising isolating free nucleic acid from a sample that contains nucleic acid wherein an agent that inhibits cell lysis is added to the sample to inhibit the lysis of cells, if cells are present wherein the agent is selected from a defined group which includes a cell lysis inhibitor.

Kiessling teach a method for preparing a sample for analysis comprising isolating free DNA (i.e. viral DNA) from a sample which comprises adding an agent that inhibits cell lysis to inhibit the lysis of cells. Note that in Kiessling the fixative utilized lyses red blood cells (i.e. RBCs) while fixing (i.e. inhibiting the lysis of) white blood cells (i.e. WBCs). See the entire document but note especially Column 2, beginning at about line

63 – Column 4 at about line 40, the first paragraph in Column 5, and Column 6, lines 3-13. Note especially Column 6, beginning at about line 40 wherein this author teaches :

> "The biological fluid sample is contacted with a disinfecting concentration of the fixative to form a fixed specimen. As used herein, the phrase "fixed specimen" has its conventional meaning known to one of ordinary skill in the art. More particularly with respect to the instant invention, "fixed specimen" refers to a biological fluid sample that has been treated to preserve the structure of cellular (e.g., leukocyte DNA) and non-cellular components (e.g., viral DNA) for analysis."

Also, Note Column 5, beginning at about line 57 wherein this author teaches:

> "In the preferred embodiments, the fixative solution contains between about 2% and about 10% paraformaldehyde. However, the preferred "disinfecting concentration" for a particular fixative is prescribed, at least in part, by the nature of the biological fluid. Thus, for blood samples in which it is desirable to simultaneously disinfect the sample, lyse the red blood cells and preserve the leukocytes for analysis, the preferred fixative is paraformaldehyde having a disinfecting concentration of between about 2% and about 4%. This is because it is generally believed that paraformaldehyde concentrations less than 2% are not immediately (i.e., within 5-10 minutes) disinfecting, while concentrations greater than 4% fix (not lyse) the red blood cells."

While this author does not explicitly teach an assay wherein the viral DNA (i.e. the free DNA) --which makes up at least a portion of the fixed non-cellular component -- is analyzed, this author does makes clear that it could be analyzed. Moreover, the totality of the prior art of record would have made the analysis of said free DNA *prima facie* obvious to the ordinary artisan at the time of the invention. Regardless this limitation (i.e. that the free DNA be analyzed) is not present in Claim 87.

Claim 88 is drawn to an embodiment of the method of Claim 87 wherein the sample is obtained from a source selected from selected from a defined group which includes a human and a mammal.

Kiessling teaches these limitations. See the first paragraph in Column 5.

Claim 89 is drawn to an embodiment of the method of Claim 88 wherein the sample is obtained from a human.

Kiessling teaches this limitation. See the first paragraph in Column 5.

Claim 90 is drawn to an embodiment of the method of Claim 87 wherein the sample is obtained from a source selected from a defined group which includes blood and urine.

Kiessling explicitly teach blood, semen, urine and sputum.

Claim 91 is drawn to an embodiment of the method of Claim 87 wherein said sample is blood

Kiessling teaches this limitation. See the first paragraph in Column 5.

Claim 92 is drawn to an embodiment of the method of Claim 91 wherein the blood is from a pregnant female. **Claim 93** is drawn to an embodiment of the method of Claim 92 wherein the blood is obtained from a pregnant female sometime during her pregnancy.

Kiessling teaches a method for preparing a sample for analysis which comprises all of the limitations of Claim 92 except these authors do not teach collecting and treating blood derived from a pregnant female. However as pregnant females were known as was the desire to detect certain viral infection in these women (e.g. HIV), it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method of Kiessling wherein the blood collected and treated is from a pregnant female. The motivation to make the modification recited above would have been the desire to test pregnant women at risk of HIV infection for HIV.

Claim 95 is drawn to an embodiment of the method of Claim 87 wherein the agent is a cell lysis inhibitor.

Kiessling teaches this limitation . See for example the first paragraph in Column 5. Note especially where this authors teach that their fixative lyses red blood cells (i.e. RBCs) while fixing (i.e. inhibiting the lysis of) white blood cells (i.e. WBCs).

Claim 96 is drawn to an embodiment of the method of Claim 87 wherein

the cell lysis inhibitor is selected from a defined group which includes derivatives of formaldehyde.

Kiessling teaches this limitation . See for example Column 6, line 3-13.

Claim 97 is drawn to an embodiment of the method of Claim 96 wherein the cell lysis inhibitor is formalin

Kiessling reasonably suggest a method for preparing a sample for analysis which comprises all of the limitations of Claim 97 except these authors do not explicitly teach using formalin as the fixative, rather these authors teach the use of a paraformaldehyde solution as a fixative. However, as evidenced by at least Halling et al., see Column 4, lines 3-19, glutaraldehyde, paraformaldehyde, formalin and formaldehyde are art recognized equivalents. See also, Column 5, lines 36-56 wherein Kiessling teaches :

> "As used herein, the term "fixative" refers to an agent that is capable of preserving the structure of a biological molecule. Fixatives that are useful for the purposes of the instant invention include the well-known fixatives that are commonly used for flow cytometry and tissue fixation applications. (See, e.g., Lifson, J., et al., J. Immunol., Methods 86:143-149 (1986), the entire contents of which are incorporated by reference). These include, for example, paraformaldehyde, **formaldehyde and glutaraldehyde**. It is believed that fixatives, such as paraformaldehyde, act by crosslinking proteins, with the resulting crosslinked products stabilizing the cellular ultrastructure. (see, e.g., Aloisio, C., and Nicholson, J., J. Immunol. Methods. 128:281-285 (1990), the entire contents of which are incorporated by reference, and references cited therein). Also provided herein is a method for evaluating (screening) aldehyde and non-aldehyde (e.g., Streck's Tissue Fixative ("STF")) fixatives for use in accordance with the methods of the invention. (See e.g.,Science 260:976-979 (1993), the entire contents of which are incorporated herein by reference)."

It should be noted that formalin is simply a dilute aqueous solution of formaldehyde. In view of these findings and absent an unexpected result it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method of Kiessling wherein formalin is used in place of the paraformaldehyde of Kiessling. Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution

recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

Claim 98 is drawn to an embodiment of the method of Claim 97 wherein the final concentration of the formalin cell lysis inhibitor is selected from a defined group.

Claim 99 is drawn to an embodiment of the method of Claim 987 wherein the final concentration of the formalin in the sample is 0.1%.

Kiessling reasonably suggest a method for preparing a sample for analysis which comprises all of the limitations of Claim 98 except this author does not teach the particular concentrations recited in Claims 98-99 rather Kiessling teaches in the paragraph bridging Columns 5-6.

> "In the preferred embodiments, the fixative solution contains between about 2% and about 10% paraformaldehyde. However, the preferred "disinfecting concentration" for a particular fixative is prescribed, at least in part, by the nature of the biological fluid. Thus, for blood samples in which it is desirable to simultaneously disinfect the sample, lyse the red blood cells and preserve the leukocytes for analysis, the preferred fixative is paraformaldehyde having a disinfecting concentration of between about 2% and about 4%. This is because it is generally believed that paraformaldehyde concentrations less than 2% are not immediately (i.e., within 5-10 minutes) disinfecting, while concentrations greater than 4% fix (not lyse) the red blood cells."

In view of these findings and absent an unexpected result it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method of Kiessling wherein formalin/formaldehyde is used in place of the paraformaldehyde of Kiessling. Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose.

Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

As regards the particular formalin concentrations recited the applicant should note that where the general conditions of a claim are disclosed in the prior art, it is not inventive, in the absence of an unexpected result, to discover the optimum or workable ranges by routine experimentation. *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955).

36. Claim(s) 94 is/are rejected under 35 U.S.C. 103(a) as being unpatentable over Kiessling [US 5,618,664(1998)] as applied against Claims 93 above and further in view of Lo et al. [US 6,156,504 (2000)].

Claim 94 is drawn to an embodiment of the method of Claim 93 wherein the sample is obtained from plasma of said blood.

Kiessling teaches a method for preparing a sample for analysis which comprises all of the limitations of Claim 94 except these authors do not teach an embodiment wherein the sample is plasma from a blood sample. However, Lo et al. do teach isolating and amplifying the DNA present in blood serum. Therefore, absent an unexpected result it would have been *prima facie* obvious to the ordinary artisan at the time of the invention to modify the method of Kiessling wherein the sample to which their fixative is added is the blood plasma of a pregnant female rather than whole blood. Please note that substitution of one well known method/reagent with known properties for a second well known method/reagent with well known properties would have been *prima facie* obvious to the ordinary artisan at the time of the invention in the absence of an unexpected result. As regards the motivation to make the substitution recited above, the motivation to combine arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for

their common known purpose. Support for making this obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

CLAIM OBJECTIONS

37. Claim(s) 25, 31, 35, 40, 42 and 53-55 is/are objected to as being dependent upon a rejected base claim, but would appear to be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

RESPONSE TO APPLICANT'S AMENDMENT/ ARGUMENTS

38. Applicant's arguments with respect to the claimed invention have been fully and carefully considered but are moot in view of the new ground(s) of rejection.

CONCLUSION

39. Claim(s) 1-6, 8-12, 14-16, 18-83, 87-102, 132-146, 148-152, 181-196, and 201-208 is/are rejected and/or objected to for the reason(s) set forth above.

40. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ethan Whisenant, Ph.D. whose telephone number is (571) 272-0754. The examiner can normally be reached Monday-Friday from 8:30AM - 5:30PM EST or any time via voice mail. If repeated attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla, can be reached at (571) 272-0735.

The Central Fax number for the USPTO is (571) 273-8300. Please note that the faxing of papers must conform with the Notice to Comply published in the Official Gazette, 1096 OG 30 (November 15, 1989).

ETHAN WHISENANT
PRIMARY EXAMINER
Art Unit 1634